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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	6232.US.P1
First Inventor or Application Identifier	George G. Schlauder
Title	See 1 in Addendum
Express Mail Label No.	EL384204542US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original and a duplicate for fee processing)*
2. [X] Specification [Total Pages 121] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 22]
4. Oath or Declaration [Total Pages]
   - a. [ ] Newly executed (original or copy)
   - b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
     - i [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

NOTE FOR ITEMS 1 & 13 IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).

5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   - a. [X] Computer Readable Copy
   - b. [X] Paper Copy (identical to computer copy)
   - c. [X] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R §3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [ ] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application Status still proper and desired
14. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
15. [X] Other: Application Cover Sheet

16. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*

[ ] Continuation [ ] Divisional [X] Continuation-in-part (CIP) of prior application No 09/173,141

Prior application information: Examiner Not assigned Group / Art Unit: Not assigned

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

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Addendum

1. METHODS AND COMPOSITIONS FOR DETECTING HEPATITIS E VIRUS

Practitioner's Docket No. 6232.US.P1 ***PATENT***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: George G. Schlauder, *et al.*

Serial No.: Group No.:
Filed: December 21, 1999 Examiner:

For: METHODS AND COMPOSITIONS FOR DETECTING HEPATITIS E VIRUS

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

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I hereby state that the following *attached* papers or fees

Utility Patent Application Transmittal – 2 pages
Fee Transmittal, 1 pg. (in duplicate)
Application Cover Sheet – 1 pg.
Specification (115 pages); Claims (5 pages); Abstract (1 page); **Total: 121**
Drawings (22 pages)
Paper copy of Sequence Listing – 140 pgs.
Computer Readable Form Copy of Sequence Listing – 1 disc
Statement to Support filings and Submission in Accordance with 37 CFR 1.821 through 1.825 – 1 pg.
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are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10, on the date indicated above and is addressed to the Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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COVER SHEET

6232.US.P1

Filed: December 21, 1999

TITLE:

METHODS AND COMPOSITIONS FOR DETECTING HEPATITIS E VIRUS

The inventors of the attached Patent Application are:

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METHODS AND COMPOSITIONS FOR DETECTING HEPATITIS E VIRUS

Related Applications

This application claims priority to U.S.S.N. 09/173,141, filed October 15, 1998, now pending, which claims priority under 35 U.S.C. §119(e) to provisional application U.S.S.N. 60/061,199, filed October 15, 1997, now abandoned, the disclosures of which are incorporated by reference herein.

Field of the Invention

This invention relates generally to methods and compositions for detecting hepatitis E virus, and more particularly to methods and compositions for detecting in, or treating individuals infected with US-type and US-subtype strains of hepatitis E virus.

Background of the Invention

There are at least five major classes of hepatotropic viruses that cause inflammation of the liver (hepatitis). These viruses include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV). Although only HBV, HCV and HDV cause chronic hepatitis, all five types cause acute disease either directly or as a result of superinfection/co-infection by, for example, HBV and HDV. HEV causes symptoms of hepatitis that are similar to those of other viral agents including abdominal pain, jaundice, malaise, anorexia, dark urine, fever, nausea and vomiting (see, for example, Reyes *et al.*, "Molecular biology of non-A, non-B hepatitis agents: hepatitis C and hepatitis E viruses" in Advances in Virus Research (1991) 40: 57-102; Bradley, "Hepatitis non-A, non-B viruses become identified as hepatitis C and E viruses" in Progr. Med. Virol. (1990) 37: 101-135; Hollinger "Non-A, non-B hepatitis viruses" in Virology, Second Edition (1990), Second Edition, Raven Press, New York pp. 2239-2271; Gust *et al.*, "Report of a workshop: waterborne non-A, non-B hepatitis" J. Infect. Dis. (1987) 156: 630-635; and Krawcyznski "Hepatitis E" Hepatology (1993) 17: 932-941). Unlike the other hepatoviruses, however, HEV generally has not been perceived as being a significant cause of hepatitis in the US.

Geographic regions where HEV is endemic include eastern and northern Africa, India, Pakistan, Burma and China (Reyes *et al.* (1991) *supra*). The case fatality rate of HEV infection is estimated to be between about 0.1% to about 1.0% in the general population, where HEV is endemic, and as high as about 20% among pregnant women in developing countries. Most fatalities result from fulminant hepatitis (Reyes *et al.* (1991) *supra*). The occasional reports of infection with HEV in the US, western Europe and Japan, usually are observed in travelers returning home from visits to areas where HEV in endemic. However, there is little information pertaining to the morbidity and/or mortality of infection with HEV in the US since HEV infections are not reported to a central agency. Extensive, systematic studies have not been performed to determine the importance of HEV in US. Further, if such studies were performed, the relative importance of HEV in US (and possibly Japan and Western Europe) may continue to be underestimated unless the proper reagents are developed to conduct such a study.

The basic features of HEV is that it is a non-enveloped virus, approximately 27-30 nm in diameter possessing a positive sense, single stranded RNA genome which comprises three discontinuous open-reading frames (ORFs), referred to in the art as open reading frame 1 (ORF 1), open reading frame 2 (ORF 2), and open reading frame 3 (ORF 3). Based on the overall morphology of the virus and the size and organization of the genome, the virus is tentatively classified as a member of the Caliciviridae. The first two isolates of HEV to be identified and sequenced were obtained from Burma and from Mexico. The overall nucleic acid identity across the genome of both isolates is 76% (Reyes *et al.* (1990) *Science*, 247: 1335-1339; Tam *et al.* (1991) *Virology* 185: 120-131; Huang *et al.* (1992) *Virology* 191: 550-558). Many of the nucleotide differences were noted at the third codon position, such that the deduced similarities in amino acid sequences between the Burmese and Mexican strains of HEV were 83%, 93% and 87%, for open reading frames ORF 1, ORF 2, and ORF 3, respectively.

In the Burmese strain, there is a short non-translated region of about 27 nucleotides at the 5' end of the genome which has not been identified in the Mexican strain. ORF 1 comprises approximately 5,100 nucleotides, which encode several conserved motifs including a putative methyltransferase domain, an RNA helicase domain, a putative RNA-dependent RNA

polymerase (RDRP) domain, and a putative papain-like protease. A tripeptide sequence of Gly-Asp-Asp (GDD), found in all positive-sense RNA plant and animal viruses, is located within ORF 1 and usually signifies RDRP function. Conserved motifs suggestive of purine NTPases activity that is usually associated with cellular and viral helicases also are present in the ORF 1 sequence. There is no consistent immune response to gene products encoded in ORF 1.

The second open reading frame (ORF 2) occupies the carboxyl one-third of the viral genome. ORF 2 comprises approximately 2,000 nucleotides which encode a consensus signal peptide sequence at the amino terminus of ORF 2, and a putative capsid protein, translated in a 1+ reading frame in relation to ORF 1. Frequently, HEV infected individuals produce antibodies that react with peptides or recombinant proteins derived from ORF 2.

The third open reading frame (ORF 3) partly overlaps both ORF 1 and ORF 2, and comprises 369 nucleotides translated in the +2 reading frame in relation to ORF 1. Although the function of the protein encoded by ORF 3 is unknown, the protein is antigenic, with most HEV infected individuals producing antibodies to this protein. Accordingly, peptides or recombinant proteins derived from ORF 2 and ORF 3 may serve as serologic markers useful in diagnosing exposure to HEV.

Recently, several additional HEV isolates have been identified and compared to the Burmese and Mexican strains of HEV. Most of the recent isolates are more closely related to the Burmese strain than to the Mexican strain of HEV. Except for a brief appearance in 1986-1987, there have been no additional isolates of the Mexican strain of HEV (Velasquez *et al.* (1992) JAMA, 263: 3281-3286).

One isolate, referred to as SAR-55, recently was isolated from an HEV-infected individual from Pakistan. The SAR-55 isolate is highly related to the Burmese strain with nucleotide and amino acid identities of 94% and 99%, respectively, across the entire genome. Several other recent isolates have been made from the Chinese province of Xuar, bordering on Pakistan. These Chinese isolates were more closely related to the Pakistani strain (approximately 98% nucleotide identity) than to the Burmese strain (approximately 93% nucleotide identity).

Prior to the sequencing of the viral genome and the availability of viral-encoded recombinant proteins and synthetic peptides, HEV infection was monitored by electron microscopy and immunofluoresence. Soon after the identification of the HEV genome, specific laboratory techniques for detecting HEV infection became available including (i) specific immunoassays, for example, western blot assays and ELISA's based on recombinant proteins and/or synthetic peptides, and (ii) polymerase chain reactions (PCR), for example, reverse transcriptase PCR (RT-PCR). RT-PCR has been used successfully to detect HEV RNA in samples of stool or serum in cases of acute hepatitis infections, and in epidemics of ET-NANBH. Furthermore, by using recombinant antigens derived from the Mexican and Burmese strains of HEV, specific IgG, IgM and, in some cases, IgA antibodies to HEV have been detected in specimens obtained from ET-NANBH outbreaks in Somalia, Burma, Borneo, Tashkent, Kenya, Pakistan and Mexico. Specific IgG, and sometimes IgM antibodies to HEV have been detected in cases of acute, sporadic hepatitis in geographic regions such as Egypt, India, Tajikistan and Uzbekistan as well as in acute hepatitis cases among patients in industrialized nations (for example, US, UK, Netherlands and Japan) who traveled to areas endemic for HEV.

To date, PCR and immunoassay-based tests based on the Burmese and Mexican isolates of HEV have established that various cases of "waterborne hepatitis" were caused by HEV. The antibody tests also were important in establishing HEV as a cause of acute, sporadic hepatitis in developing nations and among travelers to regions where HEV is endemic. However, it is unclear as to how many cases of acute HEV currently go undiagnosed due to the inability of current reagents to detect exposure to all strains of HEV. Accordingly, as new isolates of HEV are identified, it is desirable to develop new compositions and methods for detecting and/or treating hepatitis caused by the new HEV strains, which heretofore remain undetectable by the currently available test kits.

Summary of the Invention

The invention is based, in part, upon the discovery of a new family of human hepatitis E viruses. The newly discovered family of hepatitis E viruses fall within a class referred to hereinafter as a US-type hepatitis E virus. Furthermore, two members of the family were

discovered in individuals living in the United States and exhibit considerable similarities when compared at the nucleotide and amino acid levels. The latter two members together belong to a subclass of the US-type hepatitis E virus, referred to hereinafter as US-subtype hepatitis E virus.

Accordingly, in one aspect, the invention provides a method for detecting the presence of a US-type or US-subtype hepatitis E virus in a test sample of interest. The method comprises the steps of (a) contacting the test sample with a binding partner that binds specifically to a marker (or target) for the virus, which if present in the sample binds to the binding partner to produce a marker-binding partner complex, and (b) detecting the presence or absence of the complex. The presence of the complex is indicative of the presence of the virus in the test sample.

In one embodiment, the marker is an anti-US-type or anti-US-subtype antibody, for example, an immunoglobulin G (IgG) or an immunoglobulin M (IgM) molecule, present in the sample of interest, and the binding partner is an isolated polypeptide chain defining an epitope that binds specifically to the marker. In such a case, it is contemplated that the test sample is a body fluid sample, for example, blood, serum or plasma, harvested from an individual under investigation. In a preferred embodiment, the polypeptide chain defining a US-type or US-subtype specific epitope is immobilized on a solid support. Thereafter, the immobilized polypeptide chain is combined with the sample under conditions that permit the marker antibody, for example, an anti-US-type or anti-US-subtype hepatitis E virus specific antibody, present in the sample to bind to the immobilized polypeptide. Thereafter, the presence or absence of bound antibody can be detected using, for example, a second antibody or an antigen binding fragment thereof, for example, an anti-human antibody or an antigen binding fragment thereof, labeled with a detectable moiety.

It is contemplated that many different US-type and US-subtype specific polypeptides may be useful as a binding partner in the practice of this embodiment of the invention. For example, in one preferred embodiment of the invention, it is contemplated that the binding partner may be at least a portion, for example, at least 5, preferably at least 8, more preferably at least 15 and even more preferably at least about 25 amino acid residues, of a polypeptide

chain selected from the group consisting of SEQ ID NOS:91, 92 and 93, including naturally occurring variants thereof, and which represent a unique amino acid sequence when compared to the corresponding amino acid sequences of members of the Burmese and Mexican families. Similarly, it is contemplated that the binding partner may be a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NOS:173, 174, or 175. In another preferred embodiment of the invention, it is contemplated that the binding partner may be at least a portion, for example, at least 5, preferably at least 8, more preferably at least 15 and even more preferably at least about 25 amino acid residues, of a polypeptide chain selected from the group consisting of SEQ ID NOS:166, 167 and 168, including naturally occurring variants thereof, and which represent a unique amino acid sequence when compared to the corresponding amino acid sequences of members of the Burmese and Mexican families. Similarly, it is contemplated that the binding partner may be a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NOS:176, 223 or 224.

In another embodiment of the invention, the marker is a polypeptide chain unique for a member of the US-type or US-subtype families of HEV, and the binding partner preferably is an isolated antibody, for example, a polyclonal or monoclonal antibody, that binds to an epitope on the marker polypeptide chain. The binding partner may be either labeled with a detectable moiety or immobilized on a solid support. For example, it is contemplated that practice of this embodiment of the invention may be facilitated by immobilizing on a solid support, a first antibody that binds a first epitope on the marker polypeptide of interest. A test sample to be analyzed then is combined with the solid support under conditions that permit the immobilized antibody to bind the marker polypeptide. Thereafter, the presence or absence of bound marker polypeptide chain may be determined using, for example, a second antibody conjugated with a detectable moiety which binds to a second, different epitope on the marker polypeptide chain.

An antibody useful in the practice of this embodiment of the invention preferably is capable of binding specifically to a polypeptide chain selected from the group consisting of SEQ ID NOS:91, 92, and 93, including naturally occurring variants thereof, and has a higher binding affinity for such a polypeptide chain relative to the corresponding sequences of members of the Burmese and Mexican families. It is contemplated that an antibody useful in

the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NOS:173 or 175. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequence set forth in SEQ. ID NOS:169 or 171 or to the regions in the Burmese and Mexican strains that correspond to SEQ ID NO:175. Similarly, it is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NOS:174 or 176. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequence set forth in SEQ. ID NOS:170 or 172 or to the regions in the Burmese and Mexican strains that correspond to SEQ ID NO:176.

Similarly, it is contemplated that an antibody useful in the practice of this embodiment of the invention preferably is capable of binding specifically to a polypeptide chain selected from the group consisting of SEQ ID NOS:166, 167, and 168, including naturally occurring variants thereof, and has a higher binding affinity for such a polypeptide chain relative to the corresponding sequences of members of the Burmese and Mexican families. It is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO: 223. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequences set forth in SEQ. ID NOS:170 or 172. Similarly, it is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:224. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequence set forth in SEQ ID NOS:169 or 171.

In another embodiment of the invention, the marker is a nucleic acid sequence defining at least a portion of a genome of a US-type or US-subtype E virus, or a sequence complementary thereto. Similarly, it is contemplated that the binding partner is an isolated nucleic acid sequence, for example, a deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or

peptidyl nucleic acid (PNA) sequence, preferably comprising 8-100 nucleotides, more preferably comprising 10 to 75 nucleotides and mostly preferably comprising 15-50 nucleotides, which is capable of hybridizing specifically, for example, under specific hybridization conditions or under specific PCR annealing conditions, to the nucleotide sequence set forth in SEQ ID NOS:89 or 164.

Practice of this embodiment of the invention may be facilitated, for example, by isolating nucleic acids from the sample of interest. Thereafter, the resulting nucleic acids, may be fractionated by, for example, gel electrophoresis, transferred to, and immobilized onto a solid support, for example, nitrocellulose or nylon membrane, or alternatively may be immobilized directly onto the solid support via conventional dot blot or slot blot methodologies. The immobilized nucleic acid then may be probed with a preselected nucleic acid sequence labeled with a detectable moiety, that hybridizes specifically to the marker sequence. Alternatively, the presence of marker nucleic acid in a sample may be determined by standard amplification based methodologies, for example, polymerase chain reaction (PCR) wherein the production of a specific amplification product is indicative of the presence of marker nucleic acid in the sample.

In another aspect, the invention provides isolated US-type and US-subtype specific polypeptides sequences. These polypeptides include those described hereinabove in the section pertaining to US-type and US-subtype hepatitis E specific polypeptides chains useful as binding partners. In a preferred embodiment, the isolated polypeptide chain comprises an amino acid sequence set forth in SEQ ID NO:93, SEQ ID NO:168, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:223 or SEQ ID NO:224. It is contemplated that these and other US-type and US-subtype specific polypeptide chains may be employed in an assay format for detecting the presence of anti-US-type of US-subtype hepatitis E specific antibodies in a sample. In addition, it is contemplated that these polypeptides may be used either alone or in combination with adjuvants for the production of antibodies in laboratory animals, or similarly, used in combination with pharmaceutically acceptable carriers as vaccines for either the prophylactic or therapeutic immunization of mammals.

In another aspect, the invention provides isolated anti-US-type or anti-US-subtype

hepatitis E specific antibodies, which include those discussed hereinabove in the section pertaining to antibodies useful as binding partners. In a preferred embodiment, the isolated antibody is capable of binding specifically to a polypeptide chain selected from the group consisting of a polypeptide encoded by an ORF 1 sequence of a US-type or a US-subtype hepatitis E virus, a polypeptide encoded by an ORF 2 sequence of a US-type or a US-subtype hepatitis E virus, or a polypeptide encoded by an ORF 3 sequence of a US-type or a US-subtype hepatitis E virus. In particular, it is contemplated that useful antibodies are characterized in that they are capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:93, SEQ ID NO:168, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:223 or SEQ ID NO:224. It is contemplated that these antibodies and other antibodies may be used to advantage in immunoassays for detecting the presence in a sample of members of the US-type or US-subtype hepatitis E families. The antibody may be used either in a direct immunoassay wherein the antibody itself preferably is labeled with a detectable moiety or in an indirect immunoassay wherein the antibody itself provides a target for a second binding partner, *e.g.,* a second antibody labeled with a detectable moiety. Furthermore, it is contemplated that these antibodies may be used in combination with, for example, a pharmaceutically acceptable carrier for use in the passive, therapeutic or prophylactic immunization of a mammal.

In another aspect, the invention provides isolated nucleic acid sequences such as those discussed in the previous section pertaining to the use of nucleic acids as a marker or a binding partner for detecting the presence of a US-type or US-subtype hepatitis E virus in a sample. In a preferred embodiment, the invention provides isolated nucleic acid sequences defining at least a portion of an ORF 1, ORF 2 or ORF 3 sequence of a US-type or US-subtype hepatitis E virus, or a sequence complementary thereto. It is contemplated that these and other nucleic acid sequences may be used, for example, as nucleotide probes and/or amplification primers for detecting the presence of a US-type or US-subtype hepatitis E virus in a sample of interest. In addition, it is contemplated the nucleic acid sequences or sequences complementary thereto may be combined with a pharmaceutically acceptable carrier for use in anti-sense therapy. Furthermore, it is contemplated the nucleic acid sequences may be integrated in vectors which may then be transformed or transfected into a host cell of interest. The host cells may then be

combined with a pharmaceutically acceptable carrier and used as a vaccine, for example, a recombinant vaccine, for immunizing a mammal, either prophylactically or therapeutically, against a preselected US-type or US-subtype hepatitis E virus.

The foregoing and other objects, features and advantages of the present invention will be made more apparent from the following detailed description of preferred embodiments of the invention.

Brief Description of the Drawings

The objects and features of the invention may be better understood by reference to the drawings described below in which,

Figure 1 is a schematic representation of a HEV genome showing the relative positions of the ORF 1, ORF 2, and ORF 3 regions.

Figure 2 is a graph showing levels of serum aspartate aminotransferase (boxes) and serum total bilirubin (diamonds) in patient USP-1 from day 1 of a hospital admission through day 37 post admission.

Figure 3 is a schematic representation of the HEV US-1 genome showing the relative positions of clones isolated during the course of this work.

Figure 4 is a schematic representation of the HEV US-2 genome showing the relative positions of clones isolated during the course of this work.

Figure 5 shows an unrooted phylogenetic tree depicting the relationship of nucleotide sequences from full length HEV US-1, HEV US-2, and 10 other HEV isolates. Branch lengths are proportional to the evolutionary distances between sequences. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 100 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; and United States, US-1, US-2.

Figure 6 shows an unrooted phylogenetic tree depicting the relationship of nucleotide sequences from the ORF 2/3 regions (*i.e.,* sequences corresponding to nucleotide residue numbers 5094-7114 of SEQ ID NO:89). Branch lengths are proportional to the evolutionary distances between sequences. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 100 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; Swine, S1; and United States, US-1, US-2.

Figure 7 is a graph showing levels of alanine aminotransferase (boxes), serum aspartate transferase (circles), and gamma-glutamyltransferase (triangles) in a macaque before and after inoculation with sera harvested from patient USP-2. Also shown are times when HEV US-2 RNA were present in serum and fecal samples, as well as times when anti-HEV US-2 IgM and IgG were detectable.

Figure 8 is a schematic representation of the Itl genome showing the relative positions of clones isolated during the course of this work.

Figures 9 shows aligments of Burmese (B1), Mexican (M1), Chinese (C1), Pakistan (P1) and US-1 showing the design of HEV consensus primers for ORF 1, ORF 2/3 and ORF 2. Preferred consensus primers are denoted by the highlighted boxes.

Figure 10 shows an unrooted phylogenetic tree depicting the relationship of ORF 1 nucleotide sequences 371 nucleotides in length and corresponding to residues 26-396 of SEQ ID NO:89. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 1000 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; Italian, It1; Greek, G1, G2; and United States, US-1, US-2.

Figure 11 shows an unrooted phylogenetic tree depicting the relationship of ORF 2 nucleotide sequences 148 nucleotides in length and corresponding to residues 6307-6454 of

SEQ ID NO:89. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 1000 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; Italian, It1; Greek, G1, G2; Swine, S1; and United States, US-1 and US-2.

Figure 12 shows a schematic representation of preferred HEV-US recombinant protein constructs. In 12A, the ORF 2 and ORF 3 structural proteins of HEV are shown with the first and last amino acid positions designated. The presence of immunodominant epitopes are indicated by lines within the ORFs. Figure 12B shows an ORF 3 region that was cloned into an expression vector, with the first and last amino acid positions designated (SEQ ID NO:203 or SEQ ID NO:204). Figure 12C shows an ORF 2 region that was cloned into an expression vector, with the first and last amino acid positions designated (SEQ ID NO:199 or 200). Figure 12D shows an ORF 3/2 chimeric construct cloned into an expression vector with the first and last amino acid positions of each component of the chimeric construct designated (SEQ ID NO:206 or 207). The sequence omitted from the ORF 3/2 construct is indicated with a dashed line. In Figures 12B-12D, the presence of a FLAG® peptide at the carboxyl terminus of each construct is indicated by a solid box.

Figure 13 is a graph showing levels of alanine aminotransferase (square), IgG (circle) and IgM (star) in a macaque before and after inoculation with sera harvested from patient USP-2.

Figure 14 shows an unrooted phylogenetic tree depicting the relationship of ORF 1 nucleotide sequences 371 nucleotides in length and corresponding to residues 26-396 of SEQ ID NO:89. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 1000 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; Italian, It1; Greek, G1, G2; Austrian, Au1; Argentine, Ar1, Ar2; and United States, US-1, US-2.

Figure 15 shows an unrooted phylogenetic tree depicting the relationship of ORF 2 nucleotide sequences 148 nucleotides in length and corresponding to residues 6307-6454 of SEQ ID NO:89. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 1000 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; Italian, It1; Greek, G1, G2; Austrian, Au1; Argentine, Ar2; Swine, S1; and United States, US-1 and US-2.

Figure 16 shows an unrooted phylogenetic tree depicting the relationship of ORF 2 nucleotide sequences 98 nucleotides in length and corresponding to residues 6354-6451 of SEQ ID NO:89. The scale representing nucleotide substitutions per position is shown. The internal node numbers indicate the bootstrap values (expressed as a percentage of all trees) obtained from 1000 replicates. Isolates represented are Burmese, B1, B2; Chinese, C1, C2, C3, C4; Pakistan, P1; Indian, I1, I2; Mexican, M1; Italian, It1; Greek, G1, G2; Austrian, Au1; Argentine, Ar1, Ar2; Swine, S1; and United States, US-1 and US-2.

Detailed Description of the Invention

As mentioned above, this invention is based, in part, upon the discovery of a new family of human hepatitis E viruses. The newly discovered family of hepatitis E viruses fall within a class referred to hereinafter as a US-type hepatitis E virus. Furthermore, as mentioned above, two members of the US-type family were identified in sera obtained from two individuals living in the United States of America. These two members together belong to a subclass of the US-type hepatitis E virus, referred to hereinafter as a US-subtype hepatitis E virus. The discovery of the US-type and US-subtype hepatitis E viruses enables the development of methods and compositions for detecting the presence of a US-type of US-subtype hepatitis E virus in individuals who heretofore have not been diagnosed as suffering from hepatitis based on commercially available hepatitis detection kits, as well as methods and compositions for immunizing an individual against such a virus.

In one aspect, the invention pertains to a method of detecting the presence of a US-type or US-subtype hepatitis E virus in a test sample. The method comprises the steps of (a)

contacting the sample with a binding partner that binds specifically to a marker for such a virus, which if present in the sample binds to the binding partner to produce a marker-binding protein complex, and (b) detecting the presence or absence of the complex. The presence of the complex is indicative of the presence of the virus in the sample. Based on the discovery of the US-type and US-subtype hepatitis E virus disclosed herein, it will be apparent that a variety of assays, for example, protein- or nucleic acid-based assays, may be produced for detecting the presence of the virus in a sample. Protein-based assays may include, for example, conventional immunoassays, and nucleic acid-based assays may include, for example, conventional probe hybridization or nucleic acid sequence amplification assays, all of which are well known and thoroughly discussed in the art.

In another aspect, the invention provides reagents, for example, antibodies, epitope containing polypeptide chains, and nucleotide sequences that may be used to develop vaccines for immunizing, either prophylactically or therapeutically, an individual against a US-type or US-subtype hepatitis E virus.

I. Definitions

So that the invention may be more readily understood, certain terms as used herein are defined hereinbelow.

As used herein, the term "US-type" hepatitis E virus is understood to mean any human virus (*i.e.*, capable of infecting a human) that is serologically distinct from hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis G virus (HGV) and comprising a single stranded RNA genome defining at least one open reading frame and having a nucleotide sequence greater than 79.7% identity to the nucleotide sequence defined by residues 6307-6454 of SEQ ID NO:89.

As used herein, the term "US-subtype" hepatitis E is understood to mean any human virus (*i.e.*, capable of infecting a human) that is serologically distinct from hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis G virus (HGV) and comprising a single stranded RNA genome defining at least one

open read frame and having a nucleotide sequence greater than 90.5% identity to the nucleotide sequence defined by residues 6307-6454 of SEQ ID NO:89.

As used herein, the term, "test sample" is understood to mean any sample, for example, a biological sample, which contains the marker (for example, an antibody, antigenic protein or peptide, or nucleotide sequence) to be tested. Preferred test samples include tissue or body fluid samples isolatable from an individual under investigation. Preferred body fluid samples include, for example, blood, serum, plasma, saliva, sputum, semen, urine, feces, bile, spinal fluid, breast exude, ascities, and peritoneal fluid. Another preferred test sample is a cell line and more preferably, a mammalian cell line. A most preferred cell line is a human fetal kidney cell line.

As used herein, the term "open reading frame" or "ORF" is understood to mean a region of a polynucleotide sequence capable of encoding one or more polypeptide chains. The region may represent an entire coding sequence, *i.e.,* beginning with an initiation codon (*e.g.,* ATG (AUG)) and ending at a termination codon (*e.g.,* TAA (UAA), TAG (UAG), or TGA (UGA)), or a portion thereof.

As used herein, the term "polypeptide chain" is understood to mean any molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide chain.

As used herein, the term "epitope", as used synonymously with "antigenic determinant", is understood to mean at least a portion of an antigen capable of being specifically bound (*i.e.,* bound with an affinity greater than about 10^5 M^{-1}, and more preferably with an affinity greater than about 10^7 M^{-1}) by an antibody variable region. Conceivably, an epitope may comprise three amino acids in a spatial conformation unique to the epitope. Generally, an epitope comprises at least five amino acids, and more usually, at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope defined by the polypeptide chain. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by a competitive binding study. If a preselected antibody is immunologically reactive with a first antigen but is not immunologically reactive or is less immunologically reactive with a second, different antigen, then the two antigens are considered to be serologically distinct. As used herein, the term "affinity" is understood to mean a measure of reversible interaction between two molecules (for example, between an antibody and an antigen). The higher the affinity, the stronger the interaction between the two molecules.

As used herein, the term "detectable moiety" is understood to mean any signal generating compound, for example, chromogen, a catalyst such as an enzyme, a luminescent compound such as dioxetane, acridinium, phenanthridinium and luminol, a radioactive element, and a visually detectable label. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. Although the selection of a particular detectable moiety is not critical, the detectable moiety will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

As used herein, the term "solid support" is understood to mean any plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface. Useful surfaces include, for example, the surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cell, or duracyte. Suitable solid supports are not critical to the practice of the invention and can be selected by one skilled in the art. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. The solid support can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid support can retain an additional receptor which has the ability to attract and immobilize the capture reagent.

It is contemplated that the solid support also may comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structures generally are preferred, but materials with gel structure

in the hydrated state may be used as well. All of these materials may be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics.

Other embodiments which utilize various other solid supports also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer, described in EP Publication No. 0 326 100 and EP Publication No. 0 406 473, can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in EP Publication No. 0 273 115.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including automated and semi-automated systems wherein the solid phase comprises a microparticle (magnetic or non-magnetic). Such systems include those described in U.S. Patent Nos. 5,089,424 and 5244,630, issued February 18, 1992 and September 14, 1993, respectively.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, in particular in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test

piece which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries described in EP Publication No. 0 322 100 and EP Publication No. 0 406 473. The preferred method of attachment is by covalent attachment. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

As used herein, the terms "nucleotide sequence" or "nucleic acid sequence" is understood to mean any polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. The term refers to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modifications, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide.

As used herein, the term "primer" is understood to mean a specific oligonucleotide sequence complementary to a target nucleotide sequence which is capable of hybridizing to the target nucleotide sequence and serving as an initiation point for nucleotide polymerization catalyzed by DNA polymerase, RNA polymerase or reverse transcriptase.

When referring to a nucleic acid fragment, such a fragment is considered to "specifically hybridize" or to "specifically bind" to an HEV US-type or US-subtype polynucleotide or variants thereof, if, within the linear range of detection, the hybridization results in a stronger signal relative to the signal that would result from hybridization to an equal amount of a polynucleotide from other than an HEV US-type, US- subtype or variant thereof. A signal

which is "stronger" than another is one which is measurable over the other by the particular method of detection.

Also, when referring to a nucleic acid fragment, such a fragment is considered to hybridize under specific hybridization conditions if it specifically hybridizes under (i) typical hybridization and wash conditions, such as those described, for example, in Maniatis, (1st Edition, pages 387-389, 1982) where preferred hybridization conditions are those of lesser stringency and more preferred, higher stringency; or (ii) standard PCR conditions (Saiki, R.K. *et al.*) or "touch-down" PCR conditions (Roux, K.H., (1994), Biotechiques, 16:812-814).

As used herein, the term "probe" is understood to mean any nucleotide or nucleotide analog (*e.g.,* PNA) containing a sequence which can be used to identify specific DNA or RNA present in samples bearing the complementary sequence.

As used herein, the term "PNA" is used to mean peptide nucleic acid analog which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841, which is incorporated herein by reference. PNAs typically are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with such signal generating compounds as fluorescein, radionucleotides, chemiluminescent compounds, and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routine that PNAs or MAs can be substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

When referring to a nucleic acid fragment, such a fragment is considered to "specifically hybridize" or to "specifically bind" to an HEV US-type or US-subtype polynucleotide or

variants thereof, if, within the linear range of detection, the hybridization results in a stronger signal relative to the signal that would result from hybridization to an equal amount of a polynucleotide from other than an HEV US-type, US- subtype or variant thereof. A signal which is "stronger" than another is one which is measurable over the other by the particular method of detection.

Also, when referring to a nucleic acid fragment, such a fragment is considered to hybridize under specific hybridization conditions if it specifically hybridizes under (i) typical hybridization and wash conditions, such as those described, for example, in Maniatis, (1st Edition, pages 387-389, 1982) where preferred hybridization conditions are those of lesser stringency and more preferred, higher stringency; or (ii) standard PCR conditions (Saiki, R.K. *et al.*) or "touch-down" PCR conditions (Roux, K.H., (1994), Biotechiques, 16:812-814).

II. Detection Methods and Reagents

It is contemplated that the detection methods of the invention may employ a variety of protein-based or nucleic acid-based assays which are described in detail below.

It is contemplated that a reagent for the detection of virus or markers thereof may be either an anti-US-type and/or US-subtype hepatitis E virus antibody, a US-type and/or US-subtype specific polypeptide, or a nucleic acid defining at least a portion of the genome of a US-type and/or US-subtype hepatitis E virus or a nucleic acid sequence complementary thereto.

II. (i) Protein-based Assays

A. Marker Antibodies: It is contemplated that if the viral marker is an anti-US-type or anti-US-subtype specific antibody, for example, an IgG or an IgM, molecule circulating in the blood stream of an individual of interest, the binding partner preferably is a polypeptide defining an epitope that binds specifically to the marker.

In a preferred protocol for detecting the presence of anti-US-type or anti-US-subtype hepatitis E virus antibodies in a test sample, the protocol preferably comprises the following

steps which include: (a) providing an antigen comprising an immunologically reactive US-type or US-subtype specific polypeptide chain comprising at least 5, more preferably at least 8, even more preferably at least 15, and most preferably at least 25 contiguous amino acid residues and bindable by the antibody; (b) incubating the antigen with the test sample under conditions that permit formation of an antibody-antigen complex; and (c) detecting the presence of the complex.

It is contemplated that many, different US-type or US-subtype specific polypeptides may be useful as a binding partner for the detection of anti-US-type or anti-US-subtype antibodies. For example, it is contemplated that the polypeptide chain may be an amino acid sequence defined by SEQ ID NOS:91, 92 or 93 or an immunologically reactive fragment thereof containing, preferably at least 5, more preferably at least 8, even more preferably at least 15, and most preferably at least about 25 contiguous amino acid residues, of the polypeptide chain set forth in SEQ ID NOS:91, 92, or 93, and which represent a unique amino acid sequence when compared to the corresponding amino acid sequences of members of the Burmese and Mexican families. The Burmese family *i.e.*, "Burmese-like" strains, as used herein, presently comprises strains referred to herein as B1, B2, I1, I2, C1, C2, C3, C4 and P1 and the Mexican family presently comprises strain M1.

It is contemplated that the binding partner may be a polypeptide selected from the group consisting of polypeptides defined by SEQ ID NOS:91, 92, and 93, including naturally occurring variants thereof. As used herein the term "naturally occurring variants thereof" with respect to the polypeptide defined by SEQ ID NO:91 is understood to mean any amino acid sequence that is at least 84%, preferably at least 86%, more preferably at least 89% and even more preferably at least 95% identical to residues 1 through 1698 of SEQ ID NO:91. As used herein the term "naturally occurring variants thereof" with respect to the polypeptide defined by SEQ ID NO:92 is understood to mean any amino acid sequence that is at least 93%, preferably at least 95%, and even more preferably at least 98% identical to residues 1 through 660 of SEQ ID NO:92. As used herein the term "naturally occurring variants thereof" with respect to the polypeptide defined by SEQ ID NO:93 is understood to mean any amino acid sequence that is

at least 85.4%, preferably at least 87.4%, more preferably at least 90.4% and even more preferably at least 95% identical to residues 1 through 122 of SEQ ID NO:93.

Furthermore, it is contemplated that the binding partner may be a polypeptide encoded by a portion of an ORF 1 sequence. Proteins encoded by the ORF 1 sequence include, for example, a methyltransferase protein, a protease, a Y domain protein, an X domain protein, a helicase protein, a hypervariable region protein, and an RNA-dependent RNA polymerase protein. Accordingly, it is contemplated that a useful methyltransferase protein preferably has at least 92.3%, more preferably has at least 94.3%, and most preferably has at least 97.3% identity to residues 1-231 of SEQ ID NO:91. Also, it is contemplated that a useful protease protein preferably has at least 70.3%, more preferably has at least 72.3%, and most preferably has at least 75.3% identity to residues 424-697 of SEQ ID NO:91. Also, it is contemplated that a useful Y domain protein preferably has at least 94.6%, more preferably has at least 96.6% and most preferably has at least 99.6% identity to residues 207-424 of SEQ ID NO:91. Also it is contemplated that a useful X domain protein preferably has at least 83.4%, more preferably has at least 85.4% and most preferably has at least 88.4% identity to residues 789-947 of SEQ ID NO:91. Also, it is contemplated that a useful helicase protein has at least 92%, more preferably has at least 94% and most preferably at least 93% identity to residues 965-1197 of SEQ ID NO:91. Also, it is contemplated that a useful hypervariable region protein has at least 28.7%, more preferably has at least 30.7%, and most preferably has at least 33.7% identity to the residues 698-788 of SEQ ID NO:91. Also, it is contemplated that a useful RNA-dependent RNA polymerase has at least 88.8%, more preferably has at least 90.8%, and most preferably has at least about 93.8% identity to residues 1212-1698 of SEQ ID NO:91.

Furthermore, it is contemplated that the binding partner may be a polypeptide chain having an amino acid sequence defined by SEQ ID NOS:166, 167 or 168, or an immunologically reactive fragment thereof containing 5, preferably at least 8, more preferably at least 15 and most preferably at least 25 contiguous amino acid residues of the polypeptide chain set forth in SEQ ID NOS:166, 167 or 168, and which represent a unique amino acid sequence when compared to the corresponding amino acid sequences of members of the Burmese and Mexican families. Similarly, it is contemplated that the binding partner may be a

polypeptide selected from the group consisting of SEQ ID NOS:166, 167 and 168, including naturally occurring variants thereof. As used herein, the term "naturally occurring variants thereof" with respect to the polypeptide defined by SEQ ID NO:166 is understood to mean any amino acid sequence that is at least 83.9%, preferably at least 85.9%, more preferably at least 88.9%, and most preferably at least 95% identical to residues 1 through 1708 of SEQ ID NO:166. As used herein, the term "naturally occurring variants thereof" with respect to the polypeptide defined by SEQ ID NO:167 is understood to mean any amino acid sequence that is at least 93%, preferably at least 95%, and most preferably at least 98% identical to residues 1 through 660 of SEQ ID NO:167. As used herein, the term "naturally occurring variants thereof" with respect to the polypeptide defined by SEQ ID NO:168 is understood to mean any amino acid sequence that is at least 85.4%, preferably at least 87.4%, more preferably at least 90.4%, and even more preferably at least 95% identical to residues 1 through 122 of SEQ ID NO:168.

Furthermore, it is contemplated that the binding partner may be a polypeptide encoded by a portion of the HEV US-2 ORF 1, including, for example, a methyltransferase protein, a protease, a Y domain protein, an X domain protein, a helicase protein, a hypervariable region protein and an RNA-dependent RNA polymerase protein, or a variant thereof. Accordingly, it is contemplated that a useful methyltransferase protein preferably has at least 92.7%, more preferably has at least 94.7%, and most preferably has at least 97.7% identity to residues 1-240 of SEQ ID NO:166. Also, it is contemplated that a useful protease protein preferably has at least 69.6%, more preferably has at least 71.6%, and most preferably has at least 74.6% identity to residues 433-706 of SEQ ID NO:166. Also, it is contemplated that a useful Y domain protein preferably has at least 94.6%, more preferably has at least 96.6%, and most preferably has at least 99.6% identity to residues 216-433 of SEQ ID NO:166. Also it is contemplated that a useful X domain protein preferably has at least 82.8%, more preferably has at least 84.8%, and most preferably has at least 87.8% identity to residues 799-957 of SEQ ID NO:166. Also, it is contemplated that a useful helicase protein has at least 92.8%, more preferably has at least 94.8%, and most preferably has at least 97.8% identity to residues 975-1207 of SEQ ID NO:166. Also, it is contemplated that a useful hypervariable region protein has at least 27%, more preferably has at least 29%, and most preferably has at least 31%

identity to the residues 707-798 of SEQ ID NO:166. Also, it is contemplated that a useful RNA-dependent RNA polymerase has at least 88.7%, more preferably has at least 90.7%, and most preferably has at least 93.7% identity to residues 1222-1708 of SEQ ID NO:166.

With regard to the identification of US-type or US-subtype specific epitopes, it is contemplated that one skilled in the art in possession of nucleic acid sequences defining and/or amino acid sequences encoded by at least a portion of the genome of a US-type or US-subtype hepatitis E virus can map potential epitope sites using conventional technologies well known and thoroughly discussed in the art. In addition to the use of commercially available software packages which identify potential epitope sites in a given sequence, it is possible to identify potential epitopes by comparison of amino acid sequences encoded by such a genome with sequences encoded by the genomes of other strains of HEV whose antigenic sites have already been elucidated. See, for example, U.S. Patent Nos: 5,686,239, 5,741,490 and 5,770,689. Epitopes currently identified are shown in Figure 1, and include epitopes referred to in the art as 8-5 (SEQ ID NOS:93 AND 168), 4-2 (position 90-122 of SEQ ID NOS:93 and 168), SG3 (SEQ ID NOS:175 AND 176), 3-2 (position 613-654 of SEQ ID NOS:92 and 167) and 3-2e (position 613-660 of SEQ ID NOS:92 and 167). A method for calculating antigenic index is described by Jameson and Wolf (CABIOS, 4(1), 181-186 [1988]).

For example, two epitopes of interest are discussed in detail below and are referred to as 3-2e and 4-2 which are encoded by portions of ORF 2 and ORF 3 of the hepatitis E genome, respectively. These epitopes were identified in the Burmese strains of HEV (referred to below as B 3-2e (SEQ ID NO:172) and B 4-2 (SEQ IS NO:171)), and in the Mexican strain of HEV (referred to below as M 3-2e (SEQ ID NO:170) and M 4-2 (SEQ ID NO:169)). Similar epitopes were identified in HEV US-1 based on amino acid sequence comparisons, and are referred to below as U3-2e (SEQ ID NO:174) and U4-2 (SEQ ID NO:173). Similar epitopes were identified in HEV US-2, also based on amino acid sequence comparisons, and are referred to below as US-2 3-2e (SEQ ID NO:223) and US-2 4-2 (SEQ ID NO:224).

In addition, potential epitopes may be identified using screening procedures well known and thoroughly documented in the art. For example, based on the nucleic acid sequences defining either the entire or portions of the HEV US-1 or the HEV US-2 genome, it is possible

to generate an expression library, which, after expression can be screened to identify epitopes. For example, nucleic acid fragments representative of the HEV US-1 or the HEV US-2 genome can be cloned into the lambda-gt11 expression vector to produce a lambda-gt11 library, for example, a cDNA library. The library then is screened for encoded epitopes that can bind specifically with sera derived from individuals identified as being infected with HEV US-1 or HEV US-2. See, for example, Glover (1985) in "DNA Cloning Techniques, A Practical Approach", IRL Press, pp. 49-78. Typically, about 10^6 - 10^7 phage are screened, from which positive phage are identified, purified, and then tested for specificity of binding to sera from different individuals previously infected with HEV US-1 or HEV US-2. Phage which bind selectively to antibodies present in sera or plasma from the individual are selected for additional characterization. Once identified, an amino acid sequence of interest may be produced in large scale either by use of conventional recombinant DNA methodologies or by conventional peptide synthesis methodologies, well known and thoroughly documented in the art.

b. Marker Polypeptides: It is contemplated that if the marker is a US-type or US-subtype virus or a specific polypeptide thereof, the binding partner useful in the practice of the invention preferably is an antibody, for example, a polyclonal or monoclonal antibody, that binds to an epitope on the virus or marker polypeptide. The binding partner may be either labeled with a detectable moiety or immobilized on a solid support. In particular, the antibodies useful in the practice of this embodiment preferably are capable of binding specifically to a US-type or US-subtype specific polypeptide chain preferably at least 5, more preferably at least 8, even more preferably at least 15, and most preferably at least 25 contiguous amino acid residues in length which is unique with respect to the corresponding amino acid sequence found in members of the Burmese and Mexican families.

An antibody useful in the practice of this embodiment of the invention preferably is capable of binding specifically to a polypeptide chain selected from the group consisting of SEQ ID NOS:91, 92, and 93, including naturally occurring variants thereof, and has a higher binding affinity for such a polypeptide chain relative to the corresponding sequences of members of the Burmese and Mexican families. It is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain

comprising the amino acid sequence set forth in SEQ ID NO:173 or 175. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequence set forth in SEQ. ID NOS:169 or 171 or regions in the Burmese and Mexican strains that correspond to SEQ ID NO:175. Similarly, it is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NOS:174 or 176. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequence set forth in SEQ ID NOS:170 or 172 or regions in the Burmese and Mexican strains that correspond to SEQ ID NO:176.

Similarly, it is contemplated that an antibody useful in the practice of this embodiment of the invention preferably is capable of binding specifically to a polypeptide chain selected from the group consisting of SEQ ID NOS:166, 177, and 168, including naturally occurring variants thereof, and has a higher binding affinity for such a polypeptide chain relative to the corresponding sequences of members of the Burmese and Mexican families. It is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:223. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequences set forth in SEQ. ID NOS:170 or 172. Similarly, it is contemplated that an antibody useful in the practice of the invention preferably is capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:224. This antibody being further characterized as, under similar conditions, preferably having a lower affinity for, and most preferably failing to bind the amino acid sequence set forth in SEQ ID NOS:169 or 171.

The antibodies or antigen binding fragments thereof as described herein can be provided individually to detect US-type or US-subtype specific antigens. Combinations of the antibodies (and antigen binding fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least two antibodies, both having different binding specificities to separate US-type or US-subtype specific antigens.

c. Antibody Production: It is contemplated that one skilled in the art, in possession of the nucleic acid sequences defining, or amino acid sequences encoded by at least a portion of the ORF 1, ORF 2 and/or ORF 3 sequences of a US-type or a US-subtype hepatitis E virus may be able to produce specific antibodies using techniques well known and thoroughly documented in the art. See, for example, Practical Immunology, Butt, N.R., ed., Marcel Dekker, NY, 1984. Briefly, an isolated target protein is used to raise antibodies in a xenogenic host, such as a mouse, pig, goat or other suitable mammal. Preferred antibodies are antibodies that bind specifically to an epitope on the target protein, preferably having a binding affinity greater than 10^5M^{-1}, and most preferably having a binding affinity greater than 10^7M^{-1} for that epitope. Typically, the target protein is combined with a suitable adjuvant capable of enhancing antibody production in the host, and injected into the host, for example, by intraperitoneal administration. Any adjuvant suitable for stimulating the host's immune response may be used to advantage. A commonly used adjuvant is Freund's complete adjuvant (an emulsion comprising killed and dried microbial cells, *e.g.,* from Calbiochem Corp., San Diego, CA or Gibco, Grand Island, NY). Where multiple antigen injections are desired, the subsequent injections comprise the antigen in combination with an incomplete adjuvant (*e.g.,* cell-free emulsion).

Polyclonal antibodies may be isolated from the antibody-producing host by extracting serum containing antibodies to the protein of interest. Monoclonal antibodies may be produced by isolating host cells that produce the desired antibody, fusing these cells with myeloma cells using standard procedures known in the immunology art (See for example, Kohler and Milstein, Nature (1975) 256:495), and screening for hybrid cells (hybridomas) that react specifically with the target protein and have the desired binding affinity.

In addition, it is contemplated that when small peptides are used their immunogenicity may be enhanced by coupling to solid supports. For example, an epitope or antigenic region or fragment of a polypeptide generally is relatively small, and may comprise about 8 to 10 amino acids or less in length. Fragments of as few as 3 amino acids may characterize an antigenic region. These polypeptides may be linked to a suitable carrier molecule when the polypeptide of interest provided folds to provide the correct epitope but yet is too small to be antigenic.

Preferred linking reagents and methodologies for their use are well known in the art and may include, without limitation, N-succinimidyl-3-(2-pyrdylthio)propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-l-carboxylate (SMCC). Furthermore, polypeptides lacking sulfhydryl groups can be modified by adding a cysteine residue. These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilonamino on a lysine, or other free amino group in the other. A variety of such disulfide/amide-forming agents are known. Other bifunctional coupling agents form a thioester rather than a disulfide linkage. Many of these thioether-forming agents are commercially available and are known to those of ordinary skill in the art. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Any carrier which does not itself induce the production of antibodies harmful to the host can be used. Suitable carriers include proteins, polysaccharides such as latex functionalized sepharose, agarose, cellulose, cellulose beads, polymeric amino acids such as polyglutamic acid, polylysine, and no acid copolymers and inactive virus particles, among others. Examples of protein substrates include serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and yet other proteins known to those skilled in the art.

In addition, it is contemplated that biosynthetically produced antibody binding domains wherein the amino acid sequence of the binding domain is manipulated to enhance binding affinity to a preferred epitope also may be useful in the practice of the invention. A detailed description of their preparation can be found, for example, in Practical Immunology, Butt, W.R., ed., Marcel Dekker, New York, 1984. Optionally, a monovalent antibody fragment such as an Fab or an Fab' fragment may be utilized. Additionally, genetically engineered biosynthetic antibody binding sites may be utilized which comprise either 1) non-covalently associated or disulfide bonded synthetic V_H and V_L dimers, 2) covalently linked V_H-V_L single chain binding sites, 3) individual V_H or V_L domains, or 4) single chain antibody binding sites, as disclosed, for example, in U.S. Patent Nos. 5,091,513 and 5,132,405.

It is contemplated that intact antibodies (for example, monoclonal or polyclonal antibodies), antibody fragments or biosynthetic antibody binding sites that bind a US-type or

US-subtype hepatitis E virus specific epitope, will be useful in diagnostic and prognostic applications, and also, will be useful in passive immunotherapy.

d. Assay Formats: It is contemplated that both polypeptides which react immunologically with serum containing anti-US-type or anti-US-subtype hepatitis E virus specific antibodies, or antibodies raised against US-type or US-subtype hepatitis E specific epitopes will be useful in immunoassays to detect the presence of such a virus in a test sample of interest. Furthermore, it is contemplated that the presence of US-type or US-subtype hepatitis E virus in a sample may be detected using any of a wide range of immunoassay techniques, for example, direct assays, sandwich assays, and/or competition assays, currently known and thoroughly documented in the art. A variety of preferred assay formats are described in more detail below.

In one preferred format, the assay employs a sandwich format. Sandwich immunoassays typically are highly specific and very sensitive, provided that labels with good limits of detection are used. A detailed review of immunological assay design, theory and protocols can be found in numerous texts in the art, including Practical Immunology, Butt, W.R., ed., Marcell Dekker, New York, 1984.

In one type of sandwich format, a polypeptide (binding partner) which has been immobilized onto a solid support and is immunologically reactive with an anti-US-type or anti-US-subtype hepatitis E virus antibody (marker), is contacted with a test sample from an individual suspected of having been infected with the US-type or US-subtype hepatitis E virus, to form a mixture. The mixture then is incubated for a time and under conditions sufficient to form polypeptide/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, which specifically binds to the test sample antibody, and labeled with a detectable moiety, is contacted with the antigen/antibody complexes to form a second mixture. The second mixture then is incubated for a time and under conditions sufficient to form antigen/antibody/antibody complexes. The presence of anti-US-type or anti-US-subtype hepatitis E antibody, if any, in the test sample is determined by detecting the presence of detectable moiety immobilized to the solid support. The amount of antibody present in the test sample is proportional to the signal generated. The use of biotin

and antibiotin, biotin and avidin, biotin and streptavidin, and the like, may be used to enhance the generated signal in the assay systems described herein.

In an alternative format of the above-described assay, the immunologically reactive polypeptide may be immobilized "indirectly" to the solid support, i.e. through a monoclonal or polyclonal antibody or fragment thereof which specifically binds that polypeptide. Alternatively, in another format, the assay components may be used in the reverse configuration, such that an antibody or antigen binding fragment thereof, which specifically binds the test sample antibody, *i.e.,* marker antibody (for example, IgG or IgM) and immobilized on the solid support is contacted with the test sample, for a time and under conditions sufficient to permit formation of antibody/antibody complexes. Then, an indicator reagent, for example, a US-type or US-subtype hepatitis E polypeptide immunologically reactive with captured test sample antibody and labeled with a detectable moiety, is incubated with the antibody/antibody complexes to form a second mixture for a time and under conditions sufficient to permit formation of antibody/antibody/antigen complexes. As above, the presence of antibody in the test sample, if any, that is captured by the capture antibody or antigen binding fragment thereof immobilized on the solid support is determined by detecting the measurable signal generated by the detectable moiety.

It is contemplated that the aforementioned sandwich assays also may be used to test for the presence of a US-type or US-subtype hepatitis E virus, or immunologically reactive polypeptides thereof in a test sample by routine modification of the above-described assay configurations. It is contemplated that such modifications would be well known to one skilled in the art.

In addition to the aforementioned sandwich assays, it is contemplated that competitive assays may also be employed in the practice of the invention. In this format, one or a combination of at least two antibodies, preferably monoclonal antibodies, which specifically bind to a US-type or US-subtype hepatitis E specific polypeptide chain can be employed as a competitive probe for the detection of antibodies to the US-type or the US-subtype specific protein. For example, a first HEV US-1 specific polypeptide chain such as one of the polypeptides disclosed herein, acting as a binding partner for the marker, is immobilized on a

solid support. A test sample suspected of containing antibody to HEV US-1 antigen then is incubated with the solid support together with an indicator reagent comprising, for example, an isolated anti-US-type or anti-US-subtype antibody that binds the immobilized HEV US-1 specific polypeptide chain and labeled with a detectable moiety, for a time and under conditions sufficient to form antigen/antibody complexes immobilized to the solid support. If the marker antibody is present in the test sample, then the marker antibody competes with the labeled indicator reagent for binding the immobilized polypeptide. As the amount of marker antibody present in the test sample increases, the amount of labeled indicator reagent that binds the immobilized polypeptide decreases. A reduction in the amount of indicator reagent bound to the solid phase can be quantitated. A measurable reduction in signal compared to the signal generated from a confirmed negative non-A, non-B, non-C, non-D, non-E hepatitis test sample also is indicative of the presence of anti-HEV US-1 antibody in the test sample. It is contemplated that similar protocols may be used to identify the presence in a test sample of other hepatitis E viruses falling within the US-type or US-subtype classes.

In yet another detection method, the antibodies of the present invention may be employed to detect the presence of US-type or US-subtype hepatitis E specific antigens in fixed tissue sections, as well as fixed cells by immunohistochemical analysis. Cytochemical analysis wherein these antibodies are labeled directly with a detectable moiety (*e.g.*, fluorescein, colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled indirectly, for example, by means of a secondary antibody labeled with a detectable moiety also may be used in the practice of the invention.

In another assay format, the presence of antibody and/or antigen can be detected by means of a simultaneous assay, for example, as described in EP Publication No. 0 473 065. For example, a test sample is contacted simultaneously with (i) a capture reagent of a first analyte, wherein the capture reagent comprises a first binding member specific for a first analyte immobilized on a solid support and (ii) a capture reagent for a second analyte, wherein the capture reagent comprises a first binding member for a second analyte immobilized on a second different solid support, to produce a mixture. The mixture then is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second

analyte complexes. The complexes so-formed then are contacted with a first indicator reagent comprising a member of a binding pair specific for the first analyte labeled with a detectable moiety and a second indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a detectable moiety, to produce a second mixture. The second mixture then is incubated for a time and under conditions sufficient to produce both capture reagent/first analyte/first indicator reagent and capture reagent/second analyte/second indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated by the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample.

Other assay systems may employ an antibody which specifically binds US-type or US-subtype hepatitis E viral particles or sub-viral particles encapsulating the viral genome (or fragments thereof) by virtue of a contact between the specific antibody and the viral protein (peptide, etc.). The captured particles then can be analyzed by methods such as LCR or PCR to determine whether the viral genome is present in the test sample. The advantage of utilizing such an antigen capture amplification method is that it can separate the viral genome from other molecules in the test specimen by use of a specific antibody. Such a method has been described in EP 0 672 176, published September 20, 1995.

In general, immunoassay design considerations include preparation of antibodies (*e.g.,* monoclonal or polyclonal antibodies or antigen binding fragments thereof) having sufficiently high binding specificity for the target protein to form a complex that can be distinguished reliably from products of nonspecific interactions. Typically, the higher the antibody binding specificity, the lower the concentration of target that can be detected.

Both the polypeptide and antibody reagents of the invention may be used to develop assays as described herein to detect either the presence of an antigen from or an antibody that binds to a US-type or US-subtype hepatitis E virus. In addition to their use in immunoassays, it is contemplated that the aforementioned polypeptides may be used either alone or in combination with adjuvants for use in the production of antibodies in laboratory animals, or similarly, used in combination with pharmaceutically acceptable carriers as vaccines for either the prophylactic or therapeutic immunization of individuals. Also, it is contemplated that, in

addition to their use in immunoassays, the antibodies of the invention may be used in combination with, for example, a pharmaceutically acceptable carrier for use in passive, therapeutic or prophylactic immunization of an individual. These latter uses are described in more detail in section (III) below. The antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

Kits suitable for immunodiagnosis and containing the appropriate reagents may be constructed by packaging the appropriate materials, including, for example, a polypeptide defining a specific epitope of interest or antibodies that bind such epitopes in suitable containers. In addition, the kit optionally may include additional reagents, for example, suitable detection systems and buffers.

In addition, these antibodies, preferably monoclonal, can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of US-type or US-subtype hepatitis E specific proteins from cell cultures, or biological tissues such as blood and liver such as to purify recombinant and native viral antigens and proteins.

II. (ii) Nucleic Acid-based Assays

It is contemplated that if the marker is a US-type or US-subtype specific nucleotide sequence, the binding partner preferably also is a nucleotide sequence or an analog thereof that hybridizes specifically to the marker sequence or to regions adjacent thereto. Based on the unique polynucleotide sequences disclosed herein, it is contemplated that a binding partner may be a nucleotide sequence complementary to a US-type or US-subtype specific nucleotide sequence, for example, a nucleotide sequence or analog thereof complementary to at least a portion of an ORF 1 sequence, an ORF 2 sequence, or an ORF 3 sequence of a US-type or US-subtype hepatitis E virus, which is unique when compared to the corresponding nucleotide sequences of the Burmese and Mexican families. Furthermore, it is contemplated that noncoding portions of the genome of US-type and US-subtype hepatitis E viruses which are unique relative to the genomes of the Burmese and Mexican families of hepatitis E also may provide useful markers in the practice of the invention. Such nucleotide sequences (either

primers or probes) are of a length which allow detection of US-type or US-subtype specific sequences by hybridization and/or amplification and may be prepared using routine, standard methods, including automated oligonucleotide synthesis methodologies, well known and thoroughly discussed in the art. A complement of any unique portion of the HEV US-1 genome will be satisfactory. Complete complementarity is desirable for use as probes, although it may be unnecessary as the length of the fragment is increased.

Similarly, it is contemplated that the binding partner may be a polynucleotide sequence, for example, a DNA, RNA or PNA sequence, preferably comprising 8-100 nucleotides more preferably comprising 10-75 nucleotides and most preferably comprising 15-50 nucleotides, which is capable of hybridizing specifically to the target sequence. It is understood that the target sequence may be a nucleotide sequence defining at least a portion of a genome of a US-type or US-subtype hepatitis E virus, or a sequence complementary thereto. It is known in the art that the particular stringency conditions selected for a hybridization reaction depend largely upon the degree of complementarity of the binding partner nucleic acid sequence with the target sequence, the composition of the binding sequence and the length of the binding sequence. The parameters for determining stringency conditions are well known to those of ordinary skill in the art or are deemed to be readily ascertained from standard textbooks (see for example, Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, (Cold Spring Harbor Press, N.Y., 1989)).

The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multigene family or in related species like mouse and man.

One preferred protocol provides a method of detecting the presence or absence of a US-type or US-subtype hepatitis E virus in a test sample. The method comprises the steps of (a) providing a probe comprising a polynucleotide sequence containing at least 15 contiguous nucleotides from a US-type or US-subtype isolate, wherein the sequence is not present in other members of the hepatitis E Burmese and Mexican families; (b) contacting the test sample and the probe under conditions that permit formation of a polynucleotide duplex between the probe and its complement, in the absence of substantial polynucleotide duplex formation between the probe and non US-type and non US-subtype hepatitis polynucleotide sequences present in the test sample; and (c) detecting the presence of any polynucleotide duplexes containing the probe.

Preferred nucleotide sequences may comprise nucleotide residue numbers 1 through 5097 of SEQ ID NO:89, or a naturally occurring sequence variant thereof. With regard to this sequence, the term "a naturally occurring sequence variant" includes any nucleic acid sequence that is at least 73.3%, preferably at least 75.3%, more preferably at least 78.3%, and most preferably at least 95% identical to residues 1 through 5097 of SEQ ID NO:89. Other preferred marker or binding partner sequences may comprise nucleotide residue numbers 5132 through 7114 of SEQ ID NO:89, or a naturally occurring sequence variant thereof. With regard to this sequence, the term "naturally occurring sequence variant" includes any nucleic acid sequence that is at least 87.4%, preferably at least 89.4%, more preferably at least 92.4%, and most preferably at least 95% identical to residues 5132 through 7114 of SEQ ID NO:89. Other preferred marker or binding partner sequences may comprise nucleotide residue numbers 5094 through 5462 of SEQ ID NO:89, or a naturally occurring sequence variant thereof. With regard to this sequence, the term "naturally occurring sequence variant" includes any nucleic acid sequence that is at least 88.3% identical, preferably at least 90.3% identical, more preferably at least 93.3% identical, and most preferably at least 95% identical to residues 5094 through 5462 of SEQ ID NO:89.

Furthermore, it is contemplated that useful nucleotide sequences may include, for example, portions of the ORF 1 sequence encoding, for example, a protein selected from the group consisting of the methyltransferase protein, the protease protein, the Y domain protein, the X domain protein, the helicase protein, the hypervariable region protein and the RNA-

dependent RNA polymerase protein, or a variant thereof. Accordingly, it is contemplated that a useful methyltransferase encoding region of ORF 1 preferably has at least 78%, more preferably has at least 80%, and most preferably has at least 83% identity to residues 1-693 of SEQ ID NO:89. Also, it is contemplated that a useful protease encoding region of ORF 1 preferably has at least 66.1%, more preferably has at least 68.1%, and most preferably has at least 71.1% identity to residues 1270-2091 of SEQ ID NO:89. Also, it is contemplated that a useful Y domain encoding region of ORF 1 has at least 80%, more preferably has at least 82%, and most preferably has at least 85% identity to residues 619-1272 of SEQ ID NO:89. Also, it is contemplated that a useful X domain encoding region of ORF 1 has at least 73.5%, more preferably has at least 75.5%, and most preferably has at least 78.5% identity to residues 2365-2841 of SEQ ID NO:89. Also, it is contemplated that a useful helicase encoding region of ORF 1 has at least 77.5%, and most preferably has at least 79.5%, and most preferably has at least 81.5% identity to residues 2893-3591 of SEQ ID NO:89. Also, it is contemplated that a useful hypervariable region encoding region of ORF 1 has at least 51.2%, more preferably has at least 53.2%, and most preferably has at least 56.2% identity to residues 2092-2364 of SEQ ID NO:89. Also, it is contemplated that a useful RNA-dependent RNA polymerase encoding region of ORF 1 has at least 76.3%, more preferably has at least 78.3%, and most preferably has at least 81.3% identity to residues 3634-5094 of SEQ ID NO:89.

Preferred nucleotide sequences may comprise nucleotide residue numbers 36 through 5162 of SEQ ID NO:164, or a naturally occurring sequence variant thereof. With regard to this sequence, the term "a naturally occurring sequence variant" includes any nucleic acid sequence that is at least 73.6%, preferably at least 75.6%, more preferably at least 78.6% and more preferably at least 95% identical to residues 36 through 5162 of SEQ ID NO:164. Other preferred marker or binding partner sequences may comprise nucleotide residue numbers 5197 through 7179 of SEQ ID NO:164, or a naturally occurring sequence variant thereof. With regard to this sequence, the term "naturally occurring sequence variant" includes any nucleic acid sequence that is at least 80.7%, preferably at least 82.7%, more preferably at least 85.7% and most preferably at least95% identical to residues 5197 through 7179 of SEQ ID NO:164. Other preferred marker or binding partner sequences may comprise nucleotide residue numbers 5159 through 5527 of SEQ ID NO:164, or a naturally occurring sequence variant thereof. With

regard to this sequence, the term "naturally occurring sequence variant" includes any nucleic acid sequence that is at least 87.9% identical, preferably at least 89.9% identical, more preferably at least 92.9% identical and even more preferably at least 95% identical to residues 5159 through 5527 of SEQ ID NO:164.

Furthermore, it is contemplated that useful HEV US-2 nucleotide sequences may include, for example, portions of the ORF 1 sequence encoding, for example, at least a portion of a protein selected from the group consisting of the methyltransferase protein, the protease protein, the Y domain protein, the X domain protein, the helicase protein, the hypervariable region protein and the RNA-dependent RNA polymerase protein, or a variant thereof. Accordingly, it is contemplated that a useful methyltransferase encoding region of ORF 1 preferably has at least 79.5%, more preferably has at least 81.5%, and most preferably has at least 84.5% identity to residues 36-755 of SEQ ID NO:164. Also, it is contemplated that a useful protease encoding region of ORF 1 preferably has at least 66.1%, more preferably has at least 68.1%, and most preferably has at least 71.1% identity to residues 1332-2153 of SEQ ID NO:164. Also, it is contemplated that a useful Y domain encoding region of ORF 1 has at least 80.7%, more preferably has at least 82.7%, and most preferably has at least 85.7% identity to residues 680-1334 of SEQ ID NO:164. Also, it is contemplated that a useful X domain encoding region of ORF 1 has at least 73.7%, more preferably has at least 75.7%, and most preferably has at least 78.7% identity to residues 2430-2906 of SEQ ID NO:164. Also, it is contemplated that a useful helicase encoding region of ORF 1 has at least 76.4%, and most preferably has at least 78.4%, and most preferably has at least 81.4% identity to residues 2958-3656 of SEQ ID NO:164. Also, it is contemplated that a useful hypervariable region encoding region of ORF 1 has at least 50.4%, more preferably has at least 52.8%, and most preferably has at least 55.8% identity to residues 2154-2429 of SEQ ID NO:164. Also, it is contemplated that a useful RNA-dependent RNA polymerase encoding region of ORF 1 has at least 76.8%, more preferably has at least 78.8%, and most preferably has at least 81.8% identity to residues 3699-5159 of SEQ ID NO:164.

Other useful nucleotide sequences comprise the nucleotide sequences that encode the amino acid sequences selected from the group consisting of SEQ ID NOS:93, 168, 173, 174, 175, 176, 223, and 224 and nucleotide sequences complementary thereto.

It is contemplated that the nucleic acid sequences provided herein may be used to determine the presence of US-type or US-subtype hepatitis E virus in a test sample by conventional nucleic acid based assays, for example, by polymerase chain reaction (PCR) and/or by blot hybridization studies (described in detail below). In addition to their use in nucleic acid based assays, it is contemplated the aforementioned nucleic acid sequences may be integrated in vectors which may then be transformed or transfected into a host cell of interest, for example, vaccinia or mycobacteria. The resulting host cells may then be combined with a pharmaceutically acceptable carrier and used, for example, as a recombinant vaccine for immunizing a mammal, either prophylactically or therapeutically, against a preselected US-type or US-subtype hepatitis E virus.

The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers typically are employed in excess to hybridize at the outside ends of complementary strands of the target nucleic acid. The primers are each extended by a polymerase, for example, a thermostable polymerase, using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then are hybridized and extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. patents 4,683,195 and 4,683,202.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess of the target nucleic acid sequence. The first probe hybridizes to a first segment of the target strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3'hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In

addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. The ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more completely in EP-A- 320 308 to K. Backman published June 16, 1989 and EP-A-439 182 to K. Backman *et al*, published July 31, 1991.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770; or to reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall, *et al.*, PCR Methods and Applications 4: 80-84 (1994).

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described in Proc. Natl. Acad. Sci. USA 87: 1874-1878 (1990) and also described in Nature 350 (No. 6313): 91-92 (1991); Q-beta amplification as described in published EP 4544610; strand displacement amplification (as described in G. T. Walker *et al.*, Clin. Chem. 42: 9-13 [1996]) and EP 684315; and target mediated amplification, as described by PCT Publication WO 9322461.

In one embodiment, the present invention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method herein provided are labeled with capture and detection labels wherein probes are labeled with one type of label and primers are labeled with the other type of label. Additionally, the primers and probes are selected such that the probe sequence has a lower melt temperature than the primer sequences. The amplification reagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence,

copies of the target sequence (an amplicon) are produced. The double stranded amplicon then is thermally denatured to produce single stranded amplicon members. Upon formation of the single stranded amplicon members, the mixture is cooled to allow the formation of complexes between the probes and single stranded amplicon members.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached to a directly detectable label. The conjugate becomes bound to the complexes and the conjugates presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

Test samples for detecting target sequences can be prepared using methodologies well known in the art such as by obtaining a sample and, if necessary, disrupting any cells contained therein to release target nucleic acids. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a modification thereof is employed in the preferred method, the entire target sequence is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides long, more typically in the range of between 20 and 30 nucleotides long. Preferred primer sequences typically are greater than 20 nucleotides long. The typical probe is in the range of between 10 and 25 nucleotides long more typically in

the range of between 15 and 20 nucleotides long. Preferred probe sequences typically are greater than 15 nucleotides long.

Alternatively, a probe may be involved in the amplifying a target sequence, via a process known as "nested PCR". In nested PCR, the probe has characteristics which are similar to those of the first and second primers normally used for amplification (such as length, melting temperature etc.) and as such, may itself serve as a primer in an amplification reaction. Generally in nested PCR, a first pair of primers (P_1 and P_2) are employed to form primary extension products. One of the primary primers (for example, P_1) may optionally be a capture primer (i.e. linked to a member of a first reactive pair), whereas the other primary primer (P_2) is not. A secondary extension product is then formed using a probe ($P_{1'}$) and a probe ($P_{2'}$) which may also have a capture type label (such as a member of a second reactive pair) or a detection label at its 5' end. The probes are complementary to and hybridize at a site on the template near or adjacent the site where the 3' termini of P_1 and P_2 would hybridize if still in solution. Alternatively, a secondary extension product can be formed using the P_1 primer with the probe ($P_{2'}$) or the P_2 primer with the probe ($P_{1'}$) sometimes referred to as "hemi-nested PCR". Thus, a labeled primer/probe set generates a secondary product which is shorter than the primary extension product. Furthermore, the secondary product may be detected either on the basis of its size or via its labeled ends (by detection methodologies well known to those of ordinary skill in the art). In this process, probe and primers are generally employed in equivalent concentrations.

Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known in the art. For example, it is a matter of routine experimentation to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), Dupont (Wilmington, DE), or Milligen (Bedford MA). Many methods have been described for labeling oligonucleotides such as the primers or probes of the present invention. Enzo Biochemical (New York, NY) and Clontech (Palo Alto, CA) both have described and commercialized probe labeling techniques. For

example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (Clontech, Palo Alto, CA). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (Clontech). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, WO 92/10506, published 25 June 1992 and U. S. Patent 5,290,925, issued March 1, 1994, teach methods for labeling probes at their 5' and 3' termini, respectively. In addition, WO 92/11388 published 9 July 1992 teaches methods for labeling probes at their ends. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong *et al.*, Tet. Letters 29(46): 5905-5908 (1988); or J. S. Cohen *et al.*, published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

Capture labels are carried by the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood, of course that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Generally, probe/single stranded amplicon member complexes can be detected using techniques commonly employed to perform heterogeneous immunoassays. Preferably, in this embodiment, detection is performed according to the protocols used by the commercially available Abbott LCx® instrumentation (Abbott Laboratories, Abbott Park, IL).

Other useful procedures known in the art include solution hybridization, and dot and slot blot hybridization protocols. The amount of the target nucleic acid present in a sample optionally may be quantitated by measuring the radioactivity of hybridized fragments, using standard procedures known in the art.

III. Vaccines

It is contemplated that vaccines may be prepared from one or more immunogenic polypeptides based on US-type and/or US-subtype specific protein sequences or antibodies that bind to such protein sequences. In addition, it is contemplated that vaccines also may comprise dead, live but attenuated US-type or US-subtype hepatitis E virus, or a live, recombinant vaccine comprising a heterologous host cell, for example, a vaccinia virus, expressing a US-type or US-subtype hepatitis E virus specific antigen.

With regard to the polypeptide based vaccines, the polypeptide must define at least one epitope. It is contemplated, however, that the vaccine may comprise a plurality of different epitopes which are defined by one or more polypeptide chains. Furthermore, it is contemplated that nonstructural proteins as well as structural proteins may provide protection against viral pathogenicity, even if they do not cause the production of neutralizing antibodies. Considering the above, multivalent vaccines against the US-type or US-subtype virus may comprise one or more structural proteins, and/or one or more nonstructural proteins. These immunogenic epitopes can be used in combinations, *i.e.,* as a mixture of recombinant proteins, synthetic peptides and/or polypeptides isolated from the virion; which may be co-administered at the same or administered at different time.

Methodologies for the preparation of protein or peptide based vaccines which contain at least one immunogenic peptide as an active ingredient are well known in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions. The preparation may be emulsified or the protein may be encapsulated in liposomes. The active immunogenic ingredients may be mixed with pharmacologically acceptable excipients which are compatible with the active ingredient. Suitable excipients include, without limitation, water, saline, dextrose, glycerol, ethanol or a combination thereof. The vaccine also may

contain small amounts of auxiliary substances such as wetting or emulsifying reagents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. For example, such adjuvants can include aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-DMP), N-acetyl-nomuramyl-L-alanyl-D-isoglutamine (CGP 11687, also referred to as nor-MDP), N-acetyl-muramyul-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'2'-dipalmitoyl sn-glycero-3-hydroxphosphoryloxy)-ethylamine (CGP 19835A, also referred to as MTP-PE), and RIBI (MPL + TDM + CWS) in a 2% squalene/Tween-80® emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic polypeptide containing a US-type or US-subtype specific antigenic sequence resulting from administration of this polypeptide in vaccines which also comprise various adjuvants under investigation.

The vaccines usually are administered by intravenous or intramuscular injection. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include but are not limited to polyalkylene glycols or triglycerides. Such suppositories may be formed from-mixtures containing the active ingredient in the range of from about 0.5% to about 10%, preferably, from about 1% to about 2% (w/w). Oral formulation may include excipients including, for example, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient, preferably about 25% to about 70% (w/w).

The polypeptide chains used in the vaccine may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include, for example, acid addition salts formed by the addition of inorganic acids such as hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, or other acids known to those skilled in the art. Salts formed with the free carboxyl groups also may be derived from inorganic bases such as sodium, potassium, ammonium, calcium or ferric hydroxides and the like, and organic

bases such as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine procaine, or other bases known to those skilled in the art.

Vaccines typically are administered in a way compatible with the dosage formulation, and in such amounts that will be effective prophylactically and/or therapeutically. The quantity to be administered generally ranges from about 5 μg to about 250 μg of antigen per dose, however the actual dose will depend upon the health and size of the subject, the capacity of the subject's immune system to synthesize antibodies, and the degree of protection sought. The vaccine may be given in a single or multiple dose schedule. A multiple dose is one in which a primary course of vaccination may be with one to ten separate doses, followed by other doses given at subsequent time intervals required to maintain and/or to reinforce the immune response, for example, at one to four months for a second dose, and if required by the individual, a subsequent dose(s) several months later. In addition, the dosage regimen may be determined, at least in part, by the need of the individual, and may be dependent upon the practitioner's judgment.

With regard to dead or otherwise inactivated US-type or US-subtype hepatitis E virus containing vaccines, inactivation may be facilitated using conventional methodologies well known and thoroughly documented in the art. Preferred inactivation methods include, for example, exposure to one or more of (i) organic solvents, (ii) detergents, (iii) formalin, and (iv) ionizing radiation. It is contemplated that some of the proteins in attenuated vaccines may cross-react with other known viruses, and thus shared epitopes may exist between a US-type or US-subtype hepatitis E virus and other members of the HEV family (for example, members of the Burmese or Mexican families) and thus give rise to protective antibodies against one or more of the disorders caused by these pathogenic agents. Preferred formulations and modes of administration are thoroughly documented in the art and so are not discussed in detail herein. The various factors to be considered may include one or more features discussed hereinabove for the peptide based vaccines.

With regard to the live, but attenuated vaccines, it may be possible to produce attenuated virus using any of the attenuation methods known and used in the art. Briefly, attenuation may be accomplished by passage of the virus at low temperatures or by introducing

missense mutations or deletions into the viral genome. Preferred formulations and modes of administration are thoroughly documented in the art and so are not discussed in detail herein. The various factors to be considered may include one or more features discussed hereinabove for the peptide based vaccines.

With regard to live, recombinant vaccines (vector vaccines), these may be developed by incorporating into the genome of a living but harmless virus or bacterium, a gene or nucleic acid sequence encoding a US-type or US-subtype hepatitis E specific polypeptide chain defining an antigenic determinant. The resulting vector organism may then be administered to the intended host. Typically, for such a vaccine to be successful, the vector organism must be viable, and either naturally non-virulent or have an attenuated phenotype. Preferred host organisms include, vaccinia virus, adenovirus, adeno-associated virus, salmonella and mycobacteria. Live strains of vaccinia virus and mycobacteria have been administered safely to humans in the forms of the smallpox and tuberculosis (BCG) vaccines, respectively. In addition, they have been shown to express foreign proteins and exhibit little or no conversion into virulent phenotypes. Vector vaccines are capable of carrying a plurality of foreign genes or nucleic acid sequences thereby permitting simultaneous vaccination against a variety of preselected antigenic determinants. Preferred formulations and modes of administration are thoroughly documented in the art and so are not discussed in detail herein.

IV. Identification of molecules with anti-US-type or anti-US-subtype hepatitis E virus activity.

In view of the discovery of specific HEV US-type sequences, it is contemplated that one skilled in the art may be able to identify molecules which either inactivate or reduce the activity of HEV US-type specific proteins, *e.g.,* the helicase, methyltransferase, or protease proteins encoded by the ORF 1 portions of the HEV genome. An exemplary protocol for identifying molecules that inhibit the HCV protease is described in U.S. Patent No. 5,597,691, the disclosure of which is incorporated herein by reference. Although, the method pertains to the

identification of HCV protease inhibitors, it is contemplated that the same or similar protocols maybe used to identify HEV protease inhibitors, or any other protein encoded by a HEV US-type sequence.

Briefly, a method for identifying HEV protease inhibitors is as follows. Typically, a substrate is employed which mimics the proteases natural substrate, but which provides a quantifiable signal when cleaved. The signal preferably is detectable by colorimetric or fluorometric means; however, other methods such as HPLC or silica gel chromatography, nuclear magnetic resonance, and the like may also be useful. After optimum substrate and protease concentrations have been determined, candidate protease inhibitors are added one at a time to the reaction mixture at a range of concentrations. The assay conditions preferably resemble the conditions under which the protease is to be inhibited *in vivo*, *i.e.,* under physiologic pH, temperature, ionic strength, etc. Suitable inhibitors exhibit strong protease inhibition at concentrations which do not raise toxic side effects in the subject. Inhibitors which compete for binding to the protease active site may require concentrations equal to or greater than the substrate concentration, while inhibitors capable of binding irreversibly to the protease active site may be added in concentrations on the order of the enzyme concentration.

It is contemplated that the inhibitors may be organic compounds, which, for example, mimic the cleavage site recognized by the HEV protease, or alternatively, may be proteins, for example, antibodies or antibody fragments capable of binding specifically to and inactivating or reducing the activity of the HEV protease. Once identified, the protease inhibitors may be administered by a variety of methods, such as intravenously, orally, intramuscularly, intraperitoneally, bronchially, intranasally, and so forth. The preferred route of administration will depend upon the nature of inhibitor. Inhibitors prepared as organic compounds may be administered orally (which is generally preferred) if well absorbed. Protein-based inhibitors (such as most antibodies or antibody derivatives) generally are administered by parenteral routes.

Examples

Practice of the invention will be more fully understood from the following examples, which are presented herein for illustrative purposes only, and should not be construed as limiting the invention in any way. All citations to the literature, both *supra* and *infra*, including patents, patent applications and scientific publications are incorporated by reference herein, in their entirety.

Example 1 – Case study

HEV strain US-1 was identified in the serum of a patient (USP-1) suffering from acute hepatitis. The patient was a 62 year old, white male who was hospitalized in Rochester, MN after a three-week history of fever, abdominal pain, jaundice, and pruritis. Onset of signs and symptoms began two weeks after returning home following a ten day trip to San Jose, California.

His past medical history included a nephrectomy for autosomal dominant polycystic kidney disease accompanied by mild renal insufficiency, and a laparoscopic cholecystectomy for symptomatic cholelithiasis. The patient had osteoanthritis and was hypertensive. Lisinopnil therapy had been initiated three months prior to admission. Physical examination revealed an ill appearing icteric white male with an enlarged tender liver, and no asterixis. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin levels were markedly elevated at the time of hospital admission and peaked 8 days and 16 days after hospitalization, respectively (Figure 2). Lisinopril was discontinued on admission. Serologies for hepatitis A (IgM and IgG anti-HAV), hepatitis B (HBsAg, IgM and IgG anti-HBc), hepatitis C (anti-HCV), and HCV RNA were negative. Ceruloplasmin, iron, transferrin, anti-nuclear and anti-smooth muscle antibodies, toxin and drug screen were all normal. Careful questioning of the patient revealed no history of ethanol use. Abdominal ultrasound and computed tomography scan, and endoscopic retrograde cholangiopancreatogram were also normal. A liver biopsy showed a severe, acute lobular hepatitis with striking pyknotic and ballooning degeneration of

hepatocytes consistent with autoimmune, drug, or viral hepatitis.

The patient made a complete clinical recovery within 2 months, with normalization of AST, ALT, and bilirubin noted about 5 months after hospital admission. No risk factors for acquiring HEV were identified. He had not traveled outside the US for over 10 years. In the 6 weeks prior to illness onset, the only meals he reported eating that were not prepared at home were at a Mexican restaurant and a large fast food restaurant chain. He had no exposure to untreated drinking water, did not report eating raw shellfish, and had no known exposure to farm animals. None of the food handlers at the Mexican restaurant or the fast food restaurant reported foreign travel since less than 5 months from admission date and none reported signs and/or symptoms of hepatitis. No other cases of non- ABC hepatitis were reported in the county health department where the patient stayed in California, and where the patient lived in Minnesota during the period of admission. No family members had signs and/or symptoms of hepatitis either during the patient's trip to California or in the subsequent 10 weeks. Serum obtained from 6 family members in California, and from his spouse who lived with him in Minnesota over the period of interest were negative for anti-HEV by EIA.

Example 2 – Identification of unique isolate of HEV US-1

The presence of HEV was determined by RT-PCR using HEV primer sequences. Briefly, nucleic acids were isolated from 25 μL of serum from patient USP-1 as previously described (Schlauder *et al.* (1995) J. Virological Methods 46: 81-89). Ethanol precipitated nucleic acids were resuspended in 3 μL of diethyl pyrocarbonate (DEPC) treated water.

cDNA synthesis and PCR were performed using the GeneAmp RNA PCR kit from Perkin-Elmer (Norwalk, CT) in accordance with the manufacturer's instructions. RNA (1 μL) was used as a template for each 10 μL cDNA reaction. cDNA synthesis was primed with specific primers added to a final concentration of 4 μM. The subsequent amplification of cDNA was primed with oligonucleotides added to a final concentration of 0.8 to 1.0 μM. PCR was performed for 40 cycles (94°C, 20 sec; 55°C, 30 sec; 72°C, 30 sec; followed by an extension cycle of 72°C for 3 min). The initial PCR reaction (2 μL) then was used as a template for a second round of amplification using a nested set of PCR primers. PCR was performed using

the GeneAmp PCR kit from Perkin-Elmer in accordance with the manufacturer's instructions. Briefly, primers were added to a final concentration of 1 μM. The initial set of experiments used three sets of primers. Two from the 5'-end of ORF 1 based on sequences from the Burmese and Mexican strains. One set from the 3'-end of ORF 1 based on the Mexican strain sequence. The three sets of primers used were as follows:

Primer Set 1

Primer	Sequence	SEQ ID NO:
5'-ORF 1-Mexican primer C375M	CTGAACATCCCGGCCGAC	SEQ ID NO:1
PCR primer A1-350M	AGAAAGCAGCGATGGAGGA	SEQ ID NO:2
PCR primer S1-34M	GCCCACCAGTTCATTAAGGCT	SEQ ID NO:3
nested PCR primer A2-320M	TCATTAATGGAGCGTGGGTG	SEQ ID NO:4
nested PCR primer S2-55M	CCTGGCATCACTACTGCTAT	SEQ ID NO:5

Primer Set 2

Primer	Sequence	SEQ ID NO:
5'-ORF 1- Burmese cDNA primer C375	CTGAACATCACGCCCAAC	SEQ ID NO:6
PCR primer A1-350	AGGAAGCAGCGGTGGACCA	SEQ ID NO:7
PCR primer S1-34	GCCCATCAGTTTATTAAGGC	SEQ ID NO:8
nested PCR primer A2-320	TCATTTATTGAGCGGGGATG	SEQ ID NO:9
nested PCR primer S2-55	CCTGGCATCACTACTGCTAT	SEQ ID NO:10

Primer Set 3

Primer	Sequence	SEQ ID NO:
3'-ORF 1- Mexican cDNA primer M1PR6	CCATGTTCCACACCGTATTCCAGAG	SEQ ID NO:11
PCR primer S4294M	GTGTTCTACGGGGATGCTTATGACG	SEQ ID NO:12
nested PCR primer M1PF6	GACTCAGTATTCTCTGCTGCCGTGG	SEQ ID NO:13
nested PCR primer A4556	GGCTCACCAGAATGCTTCTTCCAGA	SEQ ID NO:14

The 5'-ORF 1-Burmese primers are described in Schlauder *et al.* (1993) Lancet 341: 378. Primers M1PR6 and M1PF6 are described in McCaustland *et al.* (1991) J. Virological Methods 35: 331-342. The PCR products were separated by agarose gel electrophoresis and visualized by UV irradiation after ethidium bromide staining. The resulting PCR products were hybridized to a radiolabelled probe after Southern blot transfer to a nitrocellulose filter.

Radiolabelled probes were generated from PCR products purified with the QIAEX gel extraction purification kit by Qiagen (Chatsworth, CA). Radiolabel was incorporated using the Stratgene® (La Jolla, CA) Prime-It II kit according to the manufacturer's instructions. Filters were prehybridized in Rapid-hyb buffer from Amersham (Arlington Heights, IL) for 3-5 hours, and then hybridized in Fast-Pair Hybridization Solution with 100-200 cpm/cm2 at 42°C for 15-25 hours. Filters then were washed as described in Schlauder *et al.* (1992) J. Virol. Methods 37: 189-200. Phosphorimages of the probed filters were obtained with a Molecular Dynamics Phosphorimager 425E (Sunnyvale, CA).

Ethidium bromide stained bands were detected with the primers from the 5'-end of ORF 1. However, only the primers based on the Mexican strain resulted in a nested product of the expected size of 266 base pairs. Hybridization to a probe derived from a Burmese-like strain (identity > 90%) infected patient resulted in a very weak hybridization signal to the patient USP-1 derived products relative to the signal from the Burmese positive control. These results gave the first indication that this isolate was not closely related to the Burmese isolate. No probe was available from the Mexican strain.

To confirm these results, RNA was extracted from additional serum aliquots of patient USP-1. RT-PCR was performed using the 5'-ORF 1-Mexican primers, SEQ ID NOS:1-5, as described above. Following agarose gel electrophoresis and staining with ethidium bromide, a 342 bp product was visualized in each sample. The PCR products were extracted from the agarose gel using the QIAEXII Agarose Gel Extraction Kit by Qiagen (Chatsworth, CA) and cloned into pT7 Blue T-vector plasmid by Novagen (Madison, WI). The cloned products were sequenced using the SEQUENASE VERSION 2.0 sequencing kit (USB, Cleveland, OH) in accordance with the manufacturers instructions.

The nucleotide sequences obtained from the product of the latter two samples were identical and are shown in SEQ ID NO:15. These results indicate that only the cDNA primer and primer S1 from both the Burmese and Mexican strains resulted in an ethidium bromide stainable product from the patient USP-1 samples. Only the Mexican strain based nested primers, S2 and A2 generated an ethidium bromide stainable product of the expected size.

In order to determine the degree of relatedness between the HEV US-1 isolate and other known isolates of HEV, alignments of the nucleotide and amino acid sequences were performed using the program GAP of the Wisconsin Sequence Analysis Package (Version 9), available from the Genetics Computer Group, Inc., 575 Science Drive, Madison, Wisconsin, 53711. The program employs the algorithm of Needleman and Wunsch (J. Mol. Biol. (1970) 48:443-453) to calculate the degree of similarity and identity, which are expressed as percentages between the two sequences being aligned. The gap creation and gap extension penalties were 50 and 3.0, respectively, for nucleic acid sequence alignments, and 12 and 4, respectively, for amino acid sequence comparisons.

The complete nucleotide and amino acid sequences of the two 'prototype' HEV isolates from Burma and Mexico, as well as other sequences used for analyses were obtained from GenBank, with their respective accession numbers are indicated in Table 1 below. Each of the these sequences are incorporated herein by reference.

TABLE 1

Isolate	Genbank Accession Number
Mexican (M1)	M74506
Burmese (B1)	M73218
Pakistan (P1)	M80581
Chinese (C4)	D11093

A 303 base pair sequence of HEV US-1 (homologous to residues 1-303 of SEQ ID NO:89) was compared against the homologous regions identified in the Mexican, Burmese,

Pakistani, and Chinese strains. The resulting percent identities are summarized in Table 2 below.

TABLE 2. Identity over 303 nucleic acids from the 5'-end ORF 1 product

	US-1	Mexican	Burmese	Pakistan
Mexican	77.2			
Burmese	74.9	83.2		
Pakistan	75.9	83.2	95.7	
Chinese	75.9	83.5	95.7	97.4

The results in Table 2 indicate that the fragment from the 5'-end of ORF 1 from the USP-1 isolate showed a nucleic acid identity from about 74.9 to about 77.2 % relative to other known isolates of HEV. This was less than the identity between the prototype Mexican and Burmese isolates (83.2%). These results indicate that the product likely was derived from a unique isolate of HEV not previously identified.

Example 3 – Genome Extension and Sequencing of HEV US-1

The clone obtained and sequenced as described in Example 2 (SEQ ID NO:15) hereinabove was derived from a unique HEV genome, HEV US-1. To obtain sequences from additional regions of the HEV US-1 genome, several reverse transcriptase-polymerase chain reaction (RT-PCR) walking experiments were performed.

Total nucleic acids were extracted by the procedure described in Example 2 (for SEQ ID NO:19 only) or by one of the following procedures. Aliquots (25 μL) of patient USP-1 serum were extracted using the Total Nucleic Acid Extraction procedure in accordance with the manufacturers instructions (United States Biochemical) in the presence of 10 mg yeast tRNA as carrier. Nucleic acids were precipitated and resuspended in 3.75 μL RNase/DNase free water. Alternatively, total RNA was isolated from 100 μL of serum using the ToTALLY RNA isolation kit as recommended by the manufacturer (Ambion, Inc.). The resulting RNAs were treated with DNase and column purified with reagents from S.N.A.P. Total RNA isolation kit (Invitrogen, San Diego, CA). Thereafter, RNA was precipitated with 0.1 volumes of 3M

sodium acetate, 2 μL pellet paint (Novagen) as carrier, and 2 volumes ethanol. RNA pellets were dissolved in 50 μL DEPC treated water.

RT-PCR was performed using the GeneAmp RNA PCR kit in accordance with the manufacturers instructions (Perkin-Elmer). Random hexamers were used to prime cDNA synthesis in a total volume of 25 μL except for the isolation of SEQ ID NO:19 which utilized cDNA specifically primed with primer PA2-5560 (SEQ ID NO:16), as described in Example 2 above. US1-gap was generated with specifically primed cDNA generated using RNA extracted from 12.5 μL serum equivalents, primer US1 gap-a0.5 (SEQ ID NO:46), and Superscript II (3' RACE Kit: GIBCO BRL). PCR was performed with the cDNA encompassing one-fifth of the total reaction volume (2 μL for 10 μL reaction or 5 μL for 25 μL reaction, etc.). Standard PCR was performed in the presence of 2 mM $MgCl_2$ and 0.5 to 1.0 μM of each primer. Modified reactions contained 1x PCR Buffer and 20% Q Solution (Qiagen) in accordance with the manufacturer's instructions for the isolation of SEQ ID NOS:33 and 41. Reactions used two HEV consensus primers (Table 3), one HEV consensus primer and one HEV-US-1 specific primer (Table 4), two HEV US-1 specific primers (Table 5), one HEV US-1 specific primer and one HEV US-2 (see Example 5) specific primer (Table 6), or two HEV US-2 specific primers (Table 7). Reactions were subjected to thermal cycling as follows:

SEQ ID NOS:19, 24, 27, 30, 33, 41, 44, 60, 64, 68, 73, 78, and 83 were obtained by touchdown PCR. Amplification involved 43 cycles of 94°C for 30 seconds, 55°C for 30 seconds (-0.3°C/cycle), and 72°C for 1 minute. This was followed by 10 cycles of 94°C for 30 seconds, 40°C for 30 seconds, and 72°C for 1 minute. For SEQ ID NOS:38, 49, 52, and 55, cycling involved 35 rounds of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. All amplifications were preceded by 1-2 minutes at 94°C and followed by 72°C for 5 to 10 minutes. The reactions were held at 4°C prior to agarose gel analysis.

The isolation of SEQ ID NO:19 required a second round of touch down amplification to isolate the desired product. Here, 1 μL of first round was placed into a second round 25 μL reaction. The second round amplification utilized hemi-nested primers as indicated in Table 3 by reactions 1.1.1 and 1.1.2. The isolation of SEQ ID NO:24 required a second round of nested

touch down amplification as described above and indicated in Table 4 as reactions 2.1.1 and 2.1.2. The isolation of SEQ ID NOS:38 and 49 required a second round of nested PCR (Table 5) utilizing 1 μL of first round into a 25 μL reaction as described above. The isolation of SEQ ID NOS:60, 64, 68, and 73 required nested PCR in which 1 μl of the first round was amplified in a 25 μL second round reaction (Table 6). Products SEQ ID NOS:78 and 83 were generated from two rounds of amplification (Table 7).

Agarose gel electrophoresis was performed on a fraction or all of the PCR reaction in a 0.8% to 2% agarose TAE gel in the presence of 0.2 mg/mL ethidium bromide. Products were visualized by UV irradiation and products of the desired molecular weight were excised, purified using GeneClean in accordance with the manufacturers' instructions (BIO 101, Inc.), and cloned into pT7-Blue T-Vector plasmid (Novagen) II or pGEM-T Easy Vector (Promega) in accordance with the manufacturers' instructions. Cloned products were sequenced as described in Example 2 or on a ABI Model 373 DNA Sequencer using ABI Sequencing Ready Reaction Kit as specified by the manufacturer. Results of these experiments are presented hereinbelow in Tables 3, 4, 5, 6, and 7.

TABLE 3

Reaction	Primer 1	Primer 2	Approx. Prod. Size/SEQ ID
1.1.1	SEQ ID NO:17	SEQ ID NO:16	none
1.1.2	SEQ ID NO:18	SEQ ID NO:16	251 bp/SEQ ID NO:19
1.2	SEQ ID NO:28	SEQ ID NO:29	168 bp/SEQ ID NO:30

TABLE 4

Reaction	Primer 1	Primer 2	Approx. Product Size/SEQ ID NO
2.1.1	SEQ ID NO:20	SEQ ID NO:22	none
2.1.2	SEQ ID NO:21	SEQ ID NO:23	899 bp/SEQ ID NO:24
2.2	SEQ ID NO:25	SEQ ID NO:26	846 bp/SEQ ID NO:27
2.3	SEQ ID NO:31	SEQ ID NO:32	424 bp/SEQ ID NO:33
2.4	SEQ ID NO:39	SEQ ID NO:40	460 bp/SEQ ID NO:41
2.5	SEQ ID NO:42	SEQ ID NO:43	235 bp/SEQ ID NO:44

TABLE 5

Reaction	Primer Set PCR 1	Primer Set PCR 2	Approx. Product Size/SEQ ID NO:
3.1	SEQ ID NO:34/SEQ ID NO:35	SEQ ID NO:36/SEQ. ID NO:37	1186 bp/SEQ ID NO:38
3.2	SEQ ID NO:45/SEQ ID NO:46	SEQ ID NO:47/SEQ ID NO:48	545 bp/SEQ ID NO:49
3.3	SEQ ID NO:50/SEQ ID NO:51		344 bp/SEQ ID NO:52
3.4	SEQ ID NO:53/SEQ ID NO:54		194 bp/SEQ ID NO:55

TABLE 6

Reaction	Primer Set PCR 1	Primer Set PCR 2	Approx. Product Size/SEQ ID NO:
4.1	SEQ ID NO:56/SEQ ID NO:57	SEQ ID NO:58/SEQ ID NO:59	464 bp/SEQ ID NO:60
4.2	SEQ ID NO:61/SEQ ID NO:62	SEQ ID NO:63/SEQ ID NO:62	433 bp/SEQ ID NO:64
4.3	SEQ ID NO:65/SEQ ID NO:66	SEQ ID NO:65/SEQ ID NO:67	382 bp/SEQ ID NO:68
4.4	SEQ ID NO:69/SEQ ID NO:70	SEQ ID NO:71/SEQ ID NO:72	451 bp/SEQ ID NO:73

TABLE 7

Reaction	Primer Set PCR 1	Primer Set PCR 2	Approx. Product Size/SEQ ID NO:
5.1	SEQ ID NO:74/SEQ ID NO:75	SEQ ID NO:76/SEQ ID NO:77	334 bp/SEQ ID NO:78
5.2	SEQ ID NO:79/SEQ ID NO:80	SEQ ID NO:81/SEQ ID NO:82	413 bp/SEQ ID NO:83

To obtain the sequence at the 3' end of the genome, amplification utilized the 3' RACE System of GIBCO BRL in accordance with the manufacturer's instructions. It was assumed that, as an HEV strain, the 3' end of the HEV-US-1 genome would contain a poly-adenosine tail similar to the Mexican, Burmese, and Pakistani strains. RNA extracted as described above from the equivalent of 50 μL of serum was reverse transcribed utilizing the oligo dT adapter primer 5'-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTT -3' of (SEQ ID NO:84) supplied by the manufacturer. First round PCR utilized the AUAP primer supplied 5'-GGCCACGCGTCGACTAGTAC –3' (SEQ ID NO:85) and a HEV US- specific primer (Table 8) at 0.2 mM final concentration with PCR Buffer, $MgCl_2$, and cDNA concentrations as recommended. Amplification involved 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. Amplification was preceded by a 1 minute incubation at 94°C and followed by a 72°C, 10 minute extension. A second round of amplification used 1 μL of first round in a 50 μL reaction. PCR buffer was 1X final concentration with 2 mM $MgCl_2$, and 0.5 mM of each of the primers. Primers were hemi-nested with the AUAP primer and a HEV-US-1 specific primer (Table 8). Amplification conditions were the same as first round. The products were analyzed by agarose gel electrophoresis, cloned, and sequenced as above.

TABLE 8

Reaction	Primer Set PCR 1	Primer Set PCR 2	Approx. Product Size/SEQ ID NO:
8.1	SEQ ID NO:86/SEQ ID NO:85	SEQ ID NO:87/SEQ ID NO:85	960 bp/SEQ ID NO:88

The sequences obtained from the products described in Tables 3, 4, 5, 6, 7, and 8 hereinabove, and the initial PCR product near the 5' end of the genome, SEQ ID NO:15, were assembled into contigs using the programs of the GCG package (Genetics Computer Group, Madison, WI, version 9) and a consensus sequence determined. A schematic of the assembled contig is presented in Figure 3. The HEV US-1 genome is 7202 bp in length, all of which has been sequenced (SEQ ID NO:89). This sequence was translated into three open reading frames, two of which are shown in SEQ ID NO:90 (the third ORF is positioned at nucleotide positions 5094-5462 but cannot be shown in SEQ ID NO:90 due to overlap with the other two ORFs).

The resulting translations (ORF 1, ORF 2, and ORF 3) are set forth in SEQ ID NO:91, SEQ ID NO:92, and SEQ ID NO:93, respectively.

Example 4 - Identification of unique isolate of HEV US-2

A patient from the US suffering from acute hepatitis, who tested for IgG class antibodies in the HEV EIA test, also tested positive by means of a US-1 strain-specific ELISA. This patient (USP-2) diagnosed with acute hepatitis, was a 62 year old male who was admitted to the hospital with jaundice and fatigue. Initial laboratory studies indicated an ALT of 1270 U/L (normal 0-40 U/L). Since there was a recent outbreak of hepatitis A virus (HAV) in the area, it was suspected that this individual was infected with HAV. However, the anti-HAV IgM test, HAVAB-M EIA (Abbott Laboratories) was negative as were tests for serologic markers for hepatitis B virus and hepatitis C virus. This patient's history included a visit to Cancun, Mexico, several weeks prior to the onset of his illness.

The sample from the patient then was analyzed for the presence of HEV specific sequences via PCR amplification using HEV US-1 specific PCR primers. RNA was extracted using Ultraspec as described in Example 2. Random primed cDNA synthesis was performed as described in Example 3 and PCR was performed using standard conditions as described in Example 2 with HEV US-1 specific primers SEQ ID NO:94 and SEQ ID NO:96. Nested PCR was performed with primers SEQ ID NO:95 and SEQ ID NO:97. Sequencing of the PCR product was performed as described in Example 3. The sequence of the resulting PCR product is set forth in SEQ ID NO:98. GAP analysis as described in Example 2 showed that the nucleotide sequence, SEQ ID NO:98 was 95% identical to the corresponding or homologous homologous region from HEV US-1.

Example 5 - Genome Extension and Sequencing of HEV US-2

The clone obtained and sequenced in Example 4 (SEQ ID NO:98) was derived from a HEV isolate most closely related to HEV US-1. To obtain additional regions of the HEV US-2 genome, several RT-PCR walking experiments were performed as described in Example 3.

RNA was extracted using the Total Nucleic Acid Extraction procedure (United States Biochemical). Reverse transcription was random primed using the GeneAmp RNA PCR kit (Perkin-Elmer). Standard PCR was performed in the presence of 2 mM $MgCl_2$ and 0.5 to 1.0 μM of each primer. Modified reactions contained 1x PCR Buffer and 20% Q Solution (Qiagen) for the isolation of SEQ ID NOS:129, 141 and 146. Reactions used two HEV US-1 specific primers (Table 9), one HEV US-1 specific primer and one HEV consensus primer (Table 10), one HEV US-2 specific primer and one HEV consensus primer (Table 11), two HEV US-2 specific primers (Table 12), or two Burmese, Mexican, and US derived Consensus primers (described hereinbelow, Table 13).

The products shown in SEQ ID NOS:101, 102, 105, 108, 110, 113, 117, 120, 124, 149 and 151 were obtained by touchdown PCR. Amplification involved 43 cycles of 94°C for 30 seconds, 55°C for 30 seconds (-0.3°C/cycle), and 72°C for 1 minute. This was followed by 10 cycles of 94°C for 30 seconds, 40°C for 30 seconds, and 72°C for 1 minute. Cycling involving 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute was used to amplify SEQ ID NOS:129, 132, 136, 141 and 146. All amplifications were preceded by 1-2 minutes at 94°C and followed by 72°C for 5-10 minutes. The reactions were held at 4°C prior to agarose gel analysis. Isolation of many products required a second round of nested or hemi-nested PCR as shown in Tables 9-13. In these reactions 1 μL of the PCR1 product was added to 25-50 μL of the PCR2 reaction mixture and the resulting mixture cycled as in PCR1.

Reactions were analyzed and products cloned and sequenced as described in Example 3 above. The results of these experiments are presented below in Tables 9-13.

TABLE 9

Reaction	Primer set PCR1	Primer set PCR2	Approx. Product Size/SEQ ID NO:
7.1	SEQ ID NO:99/SEQ ID NO:100		331 bp/SEQ ID NO:101
7.2	SEQ ID NO:34/SEQ ID NO.:35	SEQ ID NO:36/SEQ ID NO.:37	1186 bp/SEQ ID NO:102
7.3	SEQ ID NO:103/SEQ ID NO:104		130bp/SEQ ID NO:105
7.4	SEQ ID NO:106/SEQ ID NO:107	SEQ ID NO:39/SEQ ID NO:107	564 bp/SEQ ID NO:108
7.5	SEQ ID NO.: 86/SEQ ID NO:109	SEQ ID NO:87/SEQ ID NO:109	678 bp/SEQ ID NO:110

TABLE 10

Reaction	Primer set PCR1	Primer set PCR2	Approx. Product Size/SEQ ID NO:
8.1	SEQ ID NO:111/SEQ ID NO:112		580 bp/SEQ ID NO:113
8.2	SEQ ID NO:114/SEQ ID NO:116	SEQ ID NO:116/SEQ ID NO:115	734 bp/SEQ ID NO:117

TABLE 11

Reaction	Primer set PCR1	Primer set PCR2	Approx. Product Size/ SEQ ID NO:
9.1	SEQ ID NO:118/SEQ ID NO:119		483 bp/SEQ ID NO:120
9.2	SEQ ID NO:121/SEQ ID NO:122	SEQ ID NO:121/SEQ ID NO:123	431 bp/SEQ ID NO:124
9.3	SEQ ID NO:125/SEQ ID NO:126	SEQ ID NO:127/SEQ ID NO:128	1020 bp/SEQ ID NO:129

TABLE 12

Reaction	Primer set PCR1	Primer set PCR2	Approx. Product Size/SEQ ID NO.:
10.1	SEQ ID NO:130/SEQ ID NO:131		407 bp/SEQ ID NO:132
10.2	SEQ ID NO:133/SEQ ID NO:134	SEQ ID NO:135/SEQ ID NO:134	547 bp/SEQ ID NO:136
10.3	SEQ ID NO:137/SEQ ID NO:138	SEQ ID NO:139/SEQ ID NO:140	903 bp/SEQ ID NO:141
10.4	SEQ ID NO:142/SEQ ID NO:143	SEQ ID NO:144/SEQ ID NO:145	503 bp/SEQ ID NO:146

TABLE 13

Reaction	Primer set	Approx. Product Size/SEQ ID NO.:
11.1	SEQ ID NO:147/SEQ ID NO:148	418 bp/SEQ ID NO:149
11.2	SEQ ID NO:150/SEQ ID NO:126	197 bp/SEQ ID NO:151

To obtain the sequence at the 3' end of the genome, amplification utilized the 3' RACE System of GIBCO BRL in accordance with the manufacturer's instructions as described Example 3. cDNA was generated using SEQ ID NO:84. PCR1 utilized primers SEQ ID NO:150 and SEQ ID NO:85. PCR2 primers were SEQ ID NO:152 and SEQ ID NO:85 (reaction 12.1). The resulting product was 901 bp (SEQ ID NO:153).

The isolation of new sequences located at the 5'-terminus of the HEV US-2 viral genome was achieved by inverse PCR (M. Zeiner and U. Gehring, *Biotechniques* 17: 1051-1053, 1994). Due to limited availability of sera from USP-1 and USP-2, fecal material from a HEV US-2 infected macaque (described in Example 9 below) was chosen as the source material. A product of 462 nucleotides was amplified from macaque fecal material from within the hypervariable/ proline rich hinge region using RNA extracted, reverse transcribed, and PCR amplified as described in Example 3 using primers SEQ ID NOS:154, 155, 156 and 157. This product (SEQ ID NO:158) was 100% identical to HEV US-2 sequences. Therefore, it is contemplated that, any sequences identified at the 5' end of the HEV genome from macaque feces should accurately represent the 5' end of the HEV US-2 genome. Total nucleic acids were

extracted from 200 μL of a 10% fecal suspension as described above. Reverse transcription reactions, which utilized HEV US specific primers (SEQ ID NO:159), were performed using a kit obtained from BMB (as described in M. Zeiner and U. Gehring, *Biotechniques, supra*), except that nucleic acids were denatured at 70°C for 5 min and then placed on ice prior to initiation of the RT reaction. Generation of double-stranded, circular cDNAs was performed as described in M. Zeiner and U. Gehring, *Biotechniques, supra.* The resulting circular cDNA molecules served as template for subsequent PCR reactions. The primers used in the first PCR reaction (PCR1) are shown in SEQ ID NOS:160 and 161. The nested primers used in the second PCR reaction (PCR 2) were as shown in SEQ ID NOS:162 and 163.

Products from PCR2 (reaction 13.1) were cloned into pGEM-EasyT Vector (Promega) and sequenced using an Applied Biosystems 373 Automated sequencer. One product of 221 nucleotides was identified as having the appropriate primers and HEV US-2 sequences, identifying 63 nucleotides upstream of known HEV US-2 sequences. Additional clones were identified with the appropriate primers and portions of this new sequence. Primer extension experiments performed on RNA from 100 μL of USP-2 serum or 100 μL of a 10% fecal suspension using the sequences shown in SEQ ID NOS:163 and 161 as primers were unsuccessful in confirming the length of this sequence. Pair-wise comparisons of the 63 nucleotides to 5' NTR sequences of Burmese-like isolates revealed identities greater than 94% suggesting that this is the true sequence of HEV US-2.

The sequences obtained from the products described in this Example and those described in Example 4 were assembled into contigs using programs in the GCG package (Genetics Computer Group, Madison, WI, version 9) and a consensus sequence determined. A schematic of the assembled contigs is presented in Figure 4. The genome of the HEV US-2 strain is 7277 bp in length, all of which has been sequenced and is set forth in SEQ ID NO:164. This sequence was translated into three open reading frames as indicated in SEQ ID NO:165, with the translation products of the ORF 1 and ORF 2 sequences only being shown (the third ORF is positioned at nucleotide positions 5159-5527 but cannot be shown within SEQ ID NO:165 due to overlap with the other two ORFs). The resulting translations of the ORF 1, ORF 2, and ORF 3 sequences are shown in SEQ ID NOS:166, 167 and 168, respectively.

Example 6 - Sequence Comparisons

Information about the degree of relatedness of viruses typically can be obtained by performing comparisons such as alignments of nucleotide and deduced amino acid sequences. Alignments of the sequences of the US isolates of HEV (*e.g.,* HEV US-1 and HEV US-2) with corresponding sequences of other isolates of HEV provide a quantitative assessment of the degree of similarity and identity between the sequences. In general, the calculation of the similarity between two amino acid sequences is based upon the degree of likeness exhibited between the side chains of an amino acid pair in an alignment. The degree of likeness is based upon the physical-chemical characteristics of the amino acid side chains, i.e. size, shape, charge, hydrogen-bonding capacity, and chemical reactivity. Thus similar amino acids possess side chains that have similar physical-chemical characteristics. The calculation of identity between two aligned amino acid or nucleotide sequences is, in general, an arithmetic calculation that counts the number of identical pairs of amino acids or nucleotides in an alignment and divides this number by the length of the sequence(s) in the alignment. The calculation of similarity between two aligned nucleotide sequences sometimes uses different values for transitions and transversions between paired (i.e. matched) nucleotides at various positions in the alignment. However, the magnitude of the similarity and identity scores between pairs of nucleotide sequences, are usually very close, i.e. within one to two percent.

The degree of similarity and identity was determined using the program GAP of the Wisconsin Sequence Analysis Package (Version 9). The gap creation and gap extension penalties were 50 and 3.0, respectively, for nucleic acid sequence alignments, and 12 and 4, respectively, for amino acid sequence comparisons.

As indicated previously, a partial identity exists between the initial 5'-end ORF 1 clone and other isolates of HEV, which supports the proposition that the HEV infection associated with patient USP-1 is due to a unique isolate of HEV. In order to more extensively determine the degree of relatedness between this isolate and other known isolates of HEV, alignments of the extended nucleotide and deduced amino acid sequences were performed.

Pair-wise nucleotide and amino acid comparisons of HEV US-1, HEV US-2, and 10 other full length HEV genomes (obtained from a publicly-available database, see Table 14) were performed, as described above, to determine the relationship of the US isolates to each other and to the known variants of HEV.

TABLE 14

Isolate	Genbank Accession Number
Mexican (M1)	M74560
Burmese (B1)	M73218
Burmese (B2)	D10330
Pakistan (P1)	M80581
Chinese (C1)	D11092
Chinese (C2)	L25547
Chinese (C3)	M94177
Chinese (C4)	D11093
Indian (I1)	X98292
Indian (I2)	X99441

Nucleotide identity across the entire genomes of US-1, US-2, B1, B2, I2, C1, C2, C3, P1, C4 and I1 strains is presented in Table 15. The nucleotide identities of ORF 1, ORF 2, and ORF 3 are shown in Tables 16, 17 and 18, respectively. Tables 17 and 18 also contain comparisons against a recently isolated swine (S1) sequence, available under GenBank accession number AF011921.

TABLE 15 - Nucleotide Identity Across Genome

	US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1
US-2	92.0										
B1	73.9	74.0									
B2	73.8	74.0	98.5								
I2	73.5	73.8	96.1	95.4							
C1	74.2	74.3	93.9	93.4	92.3						
C2	74.2	74.3	93.5	93.0	92.0	98.7					
C3	74.1	74.3	93.7	93.0	92.0	98.2	98.7				
P1	74.1	74.1	93.6	92.8	92.0	98.2	98.8	98.3			
C4	73.7	73.9	94.5	94.1	92.7	97.1	97.2	96.8	96.7		
I1	74.4	74.4	93.5	93.0	92.2	93.8	94.0	93.8	93.9	93.5	
M1	73.7	74.5	75.9	75.7	75.0	75.9	75.9	75.9	76.1	75.7	75.7

TABLE 16 - Nucleotide Identity Across ORF 1

	US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1
US-1											
US-2	92.0										
B1	71.7	71.6									
B2	71.7	71.8	98.6								
I2	71.2	71.5	95.7	95.1							
C1	72.1	72.1	93.5	93.1	91.8						
C2	72.2	72.3	93.1	92.7	91.5	98.6					
C3	71.9	72.2	93.3	92.8	91.4	98.1	98.7				
P1	72.2	72.1	93.1	92.6	91.4	98.2	99.0	98.4			
C4	71.5	71.7	94.6	94.4	92.3	96.7	98.8	96.3	96.4		
I1	72.3	72.3	93.2	92.8	91.5	93.6	94.0	93.7	93.9	93.3	
M1	72.0	72.6	73.6	73.5	72.5	73.7	73.8	73.8	73.9	73.4	73.5

TABLE 17 - Nucleotide Identity Across ORF 2

	US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1	M1
US-1												
US-2	92.2											
B1	79.2	79.6										
B2	86.4	79.4	98.5									
I2	79.0	79.5	99.2	98.4								
C1	79.3	79.5	94.4	98.4	98.4							
C2	79.2	79.4	94.3	97.8	97.8	98.9						
C3	79.3	79.4	94.4	97.8	97.8	98.9	98.4					
P1	79.0	79.3	93.8	98.1	98.7	99.7	99.2	99.2				
C4	78.8	79.3	94.0	97.8	97.8	98.9	98.4	98.4	97.4			
I1	79.4	79.7	94.1	97.6	97.3	97.9	97.0	94.0	93.7	93.9		
M1	78.0	79.3	81.1	90.1	98.5	90.6	90.1	81.0	81.4	90.3	90.3	
S1	92.0	98.9	79.8	84.6	85.4	85.4	85.1	80.2	80.1	84.8	85.1	84.6

TABLE 18 - Nucleotide Identity Across ORF 3

	US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1	M1
US-1												
US-2	96.2											
B1	87.0	86.6										
B2	86.4	86.3	99.2									
I2	86.4	86.9	97.8	99.2								
C1	87.3	86.3	99.2	98.4	98.4							
C2	86.4	86.1	98.1	97.3	97.8	98.9						
C3	86.7	85.6	98.1	97.3	97.8	98.9	98.4					
P1	87.0	86.6	98.9	98.1	98.7	99.7	99.2	99.2				
C4	86.2	85.8	98.1	97.6	97.8	98.9	98.4	98.4	99.2			
I1	86.4	86.6	97.8	97.6	97.6	97.9	97.0	97.0	97.8	97.8		
M1	84.6	85.2	87.8	90.1	89.5	90.6	90.1	90.1	90.9	90.3	90.3	
S1	94.9	96.7	85.1	84.6	85.4	85.4	85.1	84.8	85.6	84.8	85.1	84.6

In addition, the ORF 1 nucleotide sequences encoding the methyltransferase proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The methyltransferase encoding region of the HEV US-1 genome is represented by residues 1-693 of SEQ ID NO:89, whereas the methyltransferase encoding region of the HEV US-2 genome is represented by residues 36-755 of SEQ ID NO:164. The comparison results are set forth in Table 19.

TABLE 19 - Methyltransferase Region

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	93.4	77.0	75.2
US-2	-	-	78.5	76.0
M1	-	-	-	78.8

The ORF 1 nucleotide sequences encoding the Y domain proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The Y domain protein encoding region of the HEV US-1 genome is represented by residues 619-1272 of SEQ ID NO:89, whereas the Y domain protein encoding region of the HEV US-2 genome is represented by residues 680-1334 of SEQ ID NO:164. The comparison results are set forth in Table 20.

TABLE 20 - Y Domain

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	94.0	79.0	77.2
US-2	-	-	79.7	76.8
M1	-	-	-	78.3

The ORF 1 nucleotide sequences encoding the protease proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The protease protein encoding region of the HEV US-1 genome is represented by residues 1270-2091 of SEQ ID NO:89, whereas the protease protein encoding region of the HEV US-2 genome is represented by residues 1332-2153 of SEQ ID NO:164. The comparison results are set forth in Table 21.

TABLE 21 - Protease Region

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	91.8	65.1	64.0
US-2	-	-	65.1	63.1
M1	-	-	-	68.1

The ORF 1 nucleotide sequences encoding the hypervariable region were compared between each of the US-1, US-2, M1 and P1 isolates. The hypervariable region encoding region of the HEV US-1 genome is represented by residues 2092-2364 of SEQ IS NO:89, whereas the hypervariable region encoding region of the HEV US-2 genome is represented by residues 2194-2429 of SEQ ID NO:164. The comparison results are set forth in Table 22.

TABLE 22 - Hypervariable Region

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	83.9	40.3	50.2
US-2	-	-	45.8	49.8
M1	-	-	-	40.4

The ORF 1 nucleotide sequences encoding the X domain proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The X domain protein encoding region of the HEV US-1 genomes represented by residues 2365-2841 of SEQ ID NO:89, whereas the X domain probe encoding region of the HEV US-2 genome is represented by residues 2430-2906 of SEQ ID NO:164. The comparison results are set forth in Table 23.

TABLE 23 - X Domain

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	91.6	72.5	71.3
US-2	-	-	72.7	70.9
M1	-	-	-	72.9

The ORF 1 nucleotide sequences encoding the helicase proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The helicase encoding region of the HEV US-1 genomes represented by residues 2893-3591 of SEQ ID NO:89, whereas the helicase encoding region of the HEV US-2 genome is represented by residues 2958-3656 of SEQ ID NO:164. The comparison results are set forth in Table 24.

TABLE 24 - Helicase Region

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	92.8	76.5	75.2
US-2	-	-	75.4	74.1
M1	-	-	-	76.2

The ORF 1 nucleotide sequences encoding the RNA-dependent RNA polymerase proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The polymerase encoding region of the HEV US-1 genome is represented by residues 3634-5094 of SEQ ID NO:89, whereas the polymerase encoding region of the HEV US-2 genome is represented by residues 3699-5159 of SEQ ID NO:164. The comparison results are set forth in Table 25.

TABLE 25 - RNA-dependent RNA Polymerase Region

	% IDENTITY			
	US-1	US-2	M1	P1
US-1	-	93.1	72.9	75.3
US-2	-	-	73.6	75.8
M1	-	-	-	77.1

In addition, the amino acid identities/similarities of the proteins encoded by the ORF 1, ORF 2, and ORF 3 sequences of US-1, US-2, B1, B2, I2, C1, C2, C3, P1, C4 and I1 strains are shown in Tables 26, 27 and 28 respectively. In addition, Tables 27 and 28 also contain comparisons against the swine sequence (S1). In Tables 26, 27 and 28, the similarities are presented in the upper right hand halves of the tables and the identities are presented in the lower left hand halves of the tables.

TABLE 26 - Amino Acid Similarity/Identity Across ORF 1

		% SIMILARITY											
%		US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1	M1
	US-1		97.8	86.0	85.7	84.4	85.9	86.2	84.9	86.4	85.7	86.3	85.4
I	US-2	97.5		86.2	85.8	84.5	85.8	86.0	85.0	86.3	85.7	86.3	85.5
D	B1	82.4	82.6		98.7	96.8	98.4	98.5	97.1	98.5	98.1	98.2	87.0
E	B2	82.3	82.3	98.6		96.2	97.8	97.9	96.3	97.8	97.6	97.6	86.6
N	I2	80.7	80.7	96.3	95.7		96.3	96.4	95.0	96.3	95.9	95.9	85.2
T	C1	82.5	82.3	98.2	97.5	95.7		99.5	97.9	99.4	99.0	98.2	86.9
I	C2	82.8	82.6	98.4	97.8	95.9	99.4		98.2	99.6	99.2	98.4	87.0
T	C3	81.6	81.6	96.9	96.1	94.4	97.7	98.1		98.1	97.6	97.0	85.9
Y	P1	83.0	82.9	98.4	97.7	95.9	99.2	99.6	98.0		99.0	98.4	87.1
	C4	82.5	82.3	98.0	97.6	95.4	98.8	99.1	97.4	98.9		97.8	86.5
	I1	82.9	82.9	98.1	97.5	95.5	98.1	98.4	96.9	98.4	97.8		87.3
	M1	82.0	82.0	83.8	83.4	81.8	83.7	83.9	82.8	84.0	83.4	84.2	

TABLE 27 - Amino Acid Similarity/Identity Across ORF 2

		% SIMILARITY												
%		US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1	M1	S1
	US-1		98.3	93.3	93.0	93.0	93.5	93.2	92.9	93.2	92.4	92.6	91.5	97.1
I	US-2	98.0		93.3	93.0	93.3	93.3	93.3	93.0	93.3	92.6	92.7	91.7	99.1
D	B1	91.8	91.8		98.9	99.1	99.8	99.2	99.2	99.5	98.8	98.9	94.8	93.0
E	B2	91.5	91.5	98.9		98.3	99.1	98.5	98.5	98.8	98.2	98.2	94.1	92.7
N	I2	91.5	91.8	99.1	98.3		99.2	98.9	98.6	99.2	98.5	98.6	94.5	91.5
T	C1	92.0	92.0	99.7	98.9	99.1		99.4	99.1	99.7	98.9	99.1	95.0	93.2
I	C2	91.7	92.0	99.1	98.3	98.8	99.4		98.8	99.4	98.6	98.8	94.7	93.0
T	C3	91.4	91.7	99.1	98.3	98.5	99.1	98.8		99.1	98.3	98.5	94.4	92.7
Y	P1	91.7	92.0	99.4	98.6	99.1	99.7	99.4	99.1		98.9	99.1	95.0	93.0
	C4	90.9	91.2	98.6	98.0	98.4	98.9	98.6	98.3	98.9		98.3	94.2	92.3
	I1	91.1	91.4	98.5	97.7	98.2	98.8	98.5	98.2	98.8	98.0		94.7	92.4
	M1	90.1	90.6	93.2	92.4	92.9	93.3	93.0	92.9	93.3	92.6	93.0		91.2
	S1	97.7	98.9	91.7	91.4	91.9	91.8	91.7	91.4	91.7	90.9	91.1	90.2	

TABLE 28 - Amino Acid Similarity/Identity Across ORF 3

		% SIMILARITY												
		US-1	US-2	B1	B2	I2	C1	C2	C3	P1	C4	I1	M1	S1
%	US-1		96.7	85.2	84.4	85.2	85.2	83.6	85.2	85.2	83.6	85.2	79.5	93.5
	US-2	96.7		85.2	84.4	85.2	85.2	83.6	83.6	85.2	83.6	85.2	81.1	96.7
I	B1	84.4	84.4		98.4	100.0	100.0	98.4	98.4	100.0	98.4	98.4	87.0	83.7
D	B2	83.6	83.6	98.4		98.4	98.4	96.7	96.7	98.4	96.7	96.7	87.0	82.9
E	I2	84.4	84.4	100.0	98.4		100.0	98.4	98.4	100.0	98.4	98.4	87.0	83.7
N	C1	84.4	84.4	100.0	98.4	100.0		98.4	98.4	100.0	98.4	98.4	87.0	83.7
T	C2	82.8	82.8	98.4	96.7	98.4	98.4		96.7	98.4	97.6	96.7	85.4	82.1
I	C3	84.4	82.8	98.4	96.7	98.4	98.4	96.7		98.4	96.7	96.7	85.4	82.1
T	P1	84.4	84.4	100.0	98.4	100.0	100.0	98.4	98.4		98.4	98.4	87.0	83.7
Y	C4	82.8	82.8	98.4	96.7	98.4	98.4	97.6	96.7	98.4		96.7	85.4	82.1
	I1	84.4	84.4	98.4	96.7	98.4	98.4	96.7	96.7	98.4	96.7		88.6	83.7
	M1	78.7	80.3	87.0	87.0	87.0	87.0	85.4	85.4	87.0	85.4	88.6		79.7
	S1	93.5	96.7	82.9	82.1	82.9	82.9	81.3	81.3	82.9	81.3	82.9	78.9	

In addition, the ORF 1 amino acid sequences defining the methyltransferase proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The methyltransferase protein encoded by the HEV US-1 genome is represented by residues 1-231 of SEQ ID NO:91, whereas the methyltransferase protein encoded by the HEV US-2 genome is represented by residues 1-240 of SEQ ID NO:166. The comparison results are set forth in Table 29.

TABLE 29 - Methyltransferase Region

		% IDENTITY			
%		US-1	US-2	M1	P1
S					
I	US-1	-	98.7	91.3	88.7
M					
I	US-2	98.7	-	91.7	89.1
L					
A	M1	91.8	92.0	-	92.9
R					
I	P1	90.0	90.4	91.2	-
T					
Y					

The ORF 1 amino acid sequences defining the protease proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The protease protein encoded by the

HEV US-1 genome is represented by residues 424-697 of SEQ ID NO:91, whereas the protease protein encoded by the HEV US-2 genome is represented by residues 433-706 of SEQ ID NO:166. The comparison results are set forth in Table 30.

TABLE 30 - Protease Region

		% IDENTITY			
% SIMILARITY		US-1	US-2	M1	P1
	US-1	-	98.5	67.5	69.3
	US-2	97.8	-	67.1	68.6
	M1	73.3	73.3	-	76.6
	P1	74.4	74.0	72.2	-

The ORF 1 amino acid sequences defining Y domain proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The Y domain protein encoded by the HEV US-1 genome is represented by residues 207-424 of SEQ ID NO:91, whereas the Y domain protein encoded by the HEV US-2 genome is represented by residues 216-433 of SEQ ID NO:166. The comparison results are set forth in Table 31.

TABLE 31 - Y Domain

		% IDENTITY			
% SIMILARITY		US-1	US-2	M1	P1
	US-1	-	98.2	92.7	93.6
	US-2	98.2	-	92.7	93.6
	M1	94.0	94.0	-	93.1
	P1	94.5	94.5	91.7	-

The ORF 1 amino acid sequences defining the X domain proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The X domain encoded by the HEV US-1 genome is represented by residues 789-947 of SEQ ID NO:91, whereas the X domain protein encoded by the HEV US-2 genome is represented by residues 799-957 of SEQ ID NO:166. The comparison results are set forth in Table 32.

TABLE 32 - X Domain

		% IDENTITY			
% SIMILARITY		US-1	US-2	M1	P1
	US-1	-	97.5	82.4	80.5
	US-2	97.5	-	81.8	79.9
	M1	88.0	87.4	-	86.1
	P1	84.3	83.6	83.0	-

The ORF 1 amino acid sequences defining helicase proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The helicase encoded by the HEV US-1, US-2, M1 and P1 isolates. The helicase encoded by the HEV US-1 genome is represented by residues 965-1197 of SEQ ID NO:91, whereas the helicase encoded by the HEV US-2 genome is

represented by residues 975-1207 of SEQ ID NO:166. The comparison results are set forth in Table 33.

TABLE 33 - Helicase Region

		% IDENTITY			
% SIMILARITY		US-1	US-2	M1	P1
	US-1	-	99.1	89.7	91.0
	US-2	99.1	-	90.6	91.8
	M1	93.1	94.0	-	95.2
	P1	94.0	94.8	91.0	-

The ORF 1 amino acid sequence defining the hypervariable regions were compared between each end of the US-1, US-2, M1 and P1 isolates. The hypervariable region encoded by the HEV US-1 genome is represented by residues 698-788 of SEQ ID NO:91, whereas the hypervariable region encoded by the HEV US-2 genome is represented by residues 707-798 of SEQ ID NO:166. The comparison results are set forth in Table 34.

TABLE 34 - Hypervariable Region

		% IDENTITY			
% SIMILARITY		US-1	US-2	M1	P1
	US-1	-	82.4	25.0	27.7
	US-2	79.1	-	25.0	21.0
	M1	25.0	25.0	-	20.8
	P1	31.9	21.0	18.0	-

The ORF 1 amino acid sequence defining the RNA-dependent RNA polymerase proteins were compared between each of the US-1, US-2, M1 and P1 isolates. The polymerase encoded by the HEV US-1 genome is represented by residues 1212-1698 of SEQ ID NO:91, whereas the polymerase encoded by the HEV US-2 genome is represented by residues 1222-1708 of SEQ ID NO:166. The comparison results are set forth in Table 35.

TABLE 35 - RNA-dependent RNA Polymerase Domain

		% IDENTITY			
% SIMILARITY		US-1	US-2	M1	P1
	US-1	-	99.0	86.0	87.8
	US-2	99.0	-	86.2	87.7
	M1	89.7	89.9	-	92.6
	P1	91.6	91.6	89.5	-

In addition to the foregoing, several additional HEV isolates belonging to the HEV US-type family were identified during the course of this work (see, Example 13 below). The additional isolates are denoted as It1 (Italian strain), G1 (first Greek strain) and G2 (second Greek strain). Additional sequence comparisons were performed and include the It1, G1 and G2 sequences, the results of which are presented below in Tables 36 and 37. Table 36 shows the nucleotide and deduced amino acid identities between isolates of HEV over a 371 base (123 amino acids) ORF 1 fragment. The ORF 1 fragment corresponds to residues 26-396 of SEQ ID NO:89. Table 37 shows the nucleotide and deduced amino acid identities between isolates of HEV over a 148 base (49 amino acid) ORF 2 fragment. The ORF 2 fragment corresponds to residues 6307-6454 of SEQ ID NO:89. In both Tables 36 and 37, the isolates represented are Burmese (B1, B2), Chinese (C1, C2, C3, C4), Indian (I1, I2), Pakistan (P1), Mexican (M1), Swine (S1), United States (US-1, US-2), Greek (G1, G2) and Italian (It1).

Pairwise comparisons of the full length nucleotide sequences were preferred using the nucleotide sequences of the respective genomes of HEV US-1 and HEV US-2 together with the other genomes of the other HEV isolates identified in Table 14. The results of the comparison are shown in Table 15. At the nucleotide level, HEV US-1 and HEV US-2 were most closely related to each other, with 92.0% identity across the entire genome. The full length Burmese-like isolates demonstrated similar identities ranging from 92.0 to 98.8%. The US isolates were 73.5 to 74.5% identical to the Burmese-like and Mexican isolates. This is similar to the identity seen between any one Burmese-like isolate and the Mexican isolate, 75.0 to 76.1% nucleotide identity. These data indicate that the US isolates are members of a new strain variant of HEV, distinct from the Burmese and Mexican strains.

Similar degrees of identity are found when smaller portions of each genome are analyzed, such as the individual ORFs. These values are presented in Tables 16, 17 and 18 for ORF 1, ORF 2, and ORF 3, respectively. Across each region, the Burmese and Pakistani isolates demonstrate the highest degree of identity ranging from 93.1 to 98.9% identity. The Mexican isolate is distinct, with identities of 73.6 to 90.1% to the Burmese-like isolates. HEV US-1 nucleotide sequence analysis reveals a significant degree of divergence with ORF 1 sequences being less than 72% identical to the Burmese-like and Mexican isolates. Similarly, ORF 2 and ORF 3 sequences were less than 79.1% and 86.9% identical to the Burmese-like and Mexican isolates, respectively.

The variability seen at the nucleotide level is reflected in the amino acid similarity and identity of the translated open reading frames. ORF 1 is the most divergent product, potentially due to the presence of a hypervariable region. The US isolates possess 97.5% amino acid identity across this region (Table 26). This is similar to the 94.4 to 99.6% identity seen between Burmese-like ORF 1 proteins. The US ORF 1 products are 80.7 to 83.0% identical to Burmese-like and Mexican proteins (Table 26). These values are similar to those observed between any one Burmese-like isolates and the Mexican isolate, ranging from 81.8 to 84.2% identity. Amino acid similarity values are generally up to 3.5% higher than the identity value, reflecting a large number of conservative amino acid substitutions. The ORF 2 product is the most conserved, potentially due to its role as the viral capsid protein. The US ORF 2 products

are 98.0% identical to each other, while being 90.1 to 92% identical to Burmese and Mexican ORF 2 proteins (Table 27). Again, these ranges mirror those observed between Burmese isolates (97.7 to 99.7% identity). Identity between Burmese and Mexican isolates is slightly greater than that between the US variant and other variants, being 92.4 to 93.3%. Amino acid similarity across ORF 2 adds approximately 1.5% to the identity value. The ORF 3 product of HEV US-1 and HEV US-2 shared 96.7% amino acid identity. The Burmese isolates showed 96.7 to 100% amino acid identity. ORF 3 amino acid identities of the US isolates to the Burmese and Mexican isolates were 78.7 to 84.4%, slightly less than that observed between Burmese and Mexican isolates, 85.4 to 88.6% identity (Table 28). Amino acid similarity across ORF 3 was generally the same as the identity values, however, some comparisons demonstrated similarity values less than 1.0% greater than the identity value. These amino acid similarity and identity values indicate that the analysis of short amino acid sequences produce similar results to full length and partial nucleotide analyses, indicating that the US isolates are closely related and genetically distinct from previously characterized isolates of HEV.

Tables 27 and 28 also include pairwise amino acid sequence comparisons with a HEV-like isolate recently identified in swine (Meng *et al.* (1997) Proc. Natl. Acad. Sci. USA 94: 9860-9865. Only 2021 bp across the ORF 2/3 region have been characterized (GenBank Accession Number: AF011921). The US swine sequence is 92% identical to the corresponding region of HEV US-1 at the nucleotide level. It is noted that HEV US-1 is very similar at the amino acid level to the recently identified swine virus. For example, the HEV US-1 and swine strains exhibit 97.1% and 93.5% identity over the respective ORF 2 and ORF 3 sequences (Tables 27 and 28, respectively).

Partial sequences of 210 nucleotides from two HEV isolates from China referred to as G9 and G20 (Genbank Accession numbers X87306 and X87307, respectively) recently have been described in the literature by (Huang *et al.* (1995) J. Med Virology 47: 303-308). These fragments represent nucleotide sequences homologous to residue numbers 4533 to 4742 of SEQ ID NO:89. Their encoded amino acid sequences (69 amino acid residues in length) are homologous to residue numbers 1512-1580 of SEQ ID NO:91. The results from the pairwise comparisons of the nucleotide sequences and the predicted amino acid sequences of these

sequences are shown in Tables 38 and 39. Results indicate that the G9 and G20 isolates are 89% identical to one another at the nucleotide level across this region. The closely related Burmese and Pakistan isolates are 92.9% identical over this range. The US-1 isolate exhibits a 77.1 and 81.0 across this region suggesting that the US-1 isolate also is unique from these isolates. Although the G9 and G20 sequences are most closely related at the nucleotide level, the deduced amino acid translation of G20 is most similar/identical to the US sequence from the US-1 isolate (Table 38). This is most likely due to the short length of amino acids utilized in the analysis.

TABLE 38. Identity across 210 nucleotides of ORF 1

	Pak	Mex	US-1	G20	G9
Bur	92.9	74.8	75.7	78.1	76.7
Pak		75.2	76.7	78.1	76.7
Mex			77.1	75.2	71.9
US-1				81.0	77.1
G20					89.0

TABLE 39. Similarity/identity across 69 amino acids of ORF 1

	Pak	Mex	US-1	G20	G9
Bur	98.6 / 98.6	92.8 / 88.4	92.8 / 85.5	92.8 / 88.4	82.6 / 79.7
Pak		94.2 / 89.9	91.3 / 84.1	91.3 / 87.0	84.1 / 81.2
Mex			89.9 / 87.0	89.9 / 87.0	81.2 / 78.3
US-1				100 / 95.7	88.4 / 88.1
G20					88.4 / 87.0

Example 7 - Phylogenetic Analyses.

Alignments of nucleotide and amino acid sequences were performed in order to determine the phylogenetic relationships between the novel US-type isolates and other isolates of HEV. The alignments were made using the program PILEUP of the Wisconsin Sequence Analysis Package, version 9 (Genetics Computer Group, Madison, WI). Evolutionary distances between sequences were determined using the DNADIST program (Kimura 2-parameter method) with a transition-transversion ratio of 2.0 and PROTDIST (Dayhoff PAM matrix) program of the PHYLIP package, version 3.5c (Felsenstein 1993, Department of Genetics, University of Washington, Seattle). The computed distances were used for the construction of phylogenetic trees using the program FITCH (Fitch-Margoliash method). The robustness of the trees was determined by bootstrap resampling of the multiple-sequence alignments (100 sets or 1,000 sets) with the programs SEQBOOT, DNADIST, the neighbor-joining method of the program NEIGHBOR, and CONSENSE (PHYLIP package). Bootstrap values of less than 70% are regarded as not providing evidence for a phylogenetic grouping (Muerhoff *et al.*, (1997) Journal of Virology, 71: 6501-6508). The final trees were produced using RETREE (PHYLIP) with the midpoint rooting option and the graphical output was created with TREEVIEW (Page, (1996) Computer Applied Biosciences 12: 357-358), the results of which are presented in Figures 5, 6, 10, and 11.

Phylogenetic analysis with complete genomes. To more extensively determine the degree of relatedness between HEV US-1, HEV US-2, and other known isolates of HEV, nucleotide alignments were performed. The full length HEV US-1 and HEV US-2 genomes were aligned with 10 other isolates of HEV from which complete genomes are available (Table 14).

Examination of the phylogenetic distances based upon alignments of the HEV-US isolates and other isolates of HEV demonstrate that there is considerable evolutionary distance between those from the US and those from other geographical areas as determined using the DNADIST program (Kimura 2-parameter method) with a transition-transversion ratio of 2.0 (Table 40). The distances calculated also show the close relationship between the isolates originating from Asia. Within this Burmese-like group the maximum distance calculated from the full length alignment is 0.0850 nucleotide substitutions per base. The minimum distance between a

member of this group and a US isolate is 0.3322 substitutions. The Mexican strain shows similar distances to the Burmese-like group of 0.3055 to 0.3132 substitutions and 0.3322 to 0.3462 substitutions to the US isolate. The genetic distance between HEV US-1 and HEV US-2 of 0.0812 substitutions is similar to that seen between Burmese-like isolates. The relative evolutionary distances between the viral sequences analyzed are readily apparent upon inspection of the unrooted phylogenetic tree presented in Figure 5, where the branch lengths are proportional to the evolutionary distances. In the phylogenetic tree, the Burmese-like isolates, the Mexican isolate and the US isolates each represent a major branch. In addition, the branching of the prototype viruses are supported with bootstrap values of 100%. Analysis of smaller segments of the genome (e.g. ORF 1, ORF 2, or ORF 3) were individually analyzed resulting in trees analogous to those obtained with the full length sequence and shown in Figure 5. These analyses demonstrate that the HEV US isolates represent a distinct strain or variant of HEV and that HEV US-1 and HEV US-2 are as similar to each other as are the most divergent Burmese-like isolates.

TABLE 40 - Phylogenetic distances over the full length sequence

	B1	B2	C1	C2	C3	C4	I1	I2	P1	M1	US-1
B1											
B2	0.0149										
C1	0.0643	0.0697									
C2	0.0680	0.0733	0.0136								
C3	0.0663	0.0734	0.0178	0.0132							
C4	0.0574	0.0611	0.0304	0.0290	0.0329						
I1	0.0677	0.0728	0.0645	0.0625	0.0647	0.0681					
I2	0.0403	0.0477	0.0820	0.0849	0.0846	0.0776	0.0832				
P1	0.0693	0.0751	0.0178	0.0120	0.0172	0.0335	0.0633	0.0850			
M1	0.3096	0.3120	0.3086	0.3089	0.3091	0.3132	0.3120	0.3259	0.3055		
US-1	0.3406	0.3418	0.3360	0.3345	0.3367	0.3445	0.3322	0.3464	0.3363	0.3462	
US-2	0.3413	0.3408	0.3370	0.3361	0.3374	0.3445	0.3333	0.3461	0.3377	0.3367	0.0812

Comparison to ORF 2/ORF 3 from Swine HEV. In order to determine the relationship between a recently described swine-HEV and the human HEV US-1 and HEV US-2 isolates, comparisons of the nucleotide sequences across the complete ORF 2 and ORF 3 were performed using analogous regions from the 10 full length sequences utilized above (Table 14). Phylogenetic analysis produces genetic distances of 0.0799 to 0.0810 nucleotide substitutions per position between the US and swine HEV isolates (Table 41). These values are similar to those observed between the most distant Burmese-like isolates. The US and swine isolates group closely on an unrooted phylogenetic tree when the ORF 2/3 nucleotide sequences are analyzed (See, Figure 6). These isolates form a phylogenetic group distinct from the Mexican isolate and the Burmese-like isolates. These grouping are supported by bootstrap values of 100%.

TABLE 41 - Phylogenetic distances between USswine and human HEV isolates

	US-2	USswine	Burmese	Mexican
US-1	0.0799	0.0810	0.2441-0.2495	0.2671
US-2		0.0795	0.2409-0.2479	0.2486
USswine			0.2348-0.2485	0.2615
Burmese			0.0119-0.0716	0.2183-0.2248

Example 8 - HEV Serologic Studies

A. Background

Early studies indicate that epitopes useful for diagnosis of HEV infections are located near the carboxyl terminus of ORF 2 and ORF 3 of both the Burmese and Mexican strains of HEV. The two antigens from the Mexican strain, referred to hereinafter as M 3-2 and M 4-2, comprise 42 and 32 amino acids near the carboxyl terminus of ORF 2 and ORF 3, respectively (Yarbough *et al.* (1991) Journal of Virology, 65: 5790-5797). The two antigens from the Burmese strain of HEV, referred to hereinafter as B 3-2 and B 4-2 proteins, comprise 42 and 33 amino acids near the carboxyl terminus of ORF 2 and ORF 3, respectively (Yarbough *et al.* (1991) *supra*). Diagnostic tests designed to detect IgG, IgA and IgM class antibodies to HEV have been developed based on these antigenic regions. Additional HEV recombinant proteins have been generated that encompass full-length ORF 3 (Dawson *et al.* (1992) Journal of Virology Methods, 38: 175-186) or additional amino acid sequences from the ORF 2 protein (Dawson *et al.* (1993) *supra*), to potentially enhance the detection of antibodies to HEV. Comparative studies indicate that the original recombinant proteins and synthetic peptides (B4-2, B3-2, M3-2, M4-2) were as effective as the larger recombinant proteins in detecting antibodies to HEV in known cases of acute HEV infection. A licensed test to detect antibodies to HEV is manufactured by Abbott Laboratories and consists of the full length Burmese strain ORF 3 protein and the carboxyl 327 amino acids of the Burmese strain ORF 2 protein.

After initial serological studies demonstrating the utility of B 3-2, B 4-2, M 3-2 and M

4-2, it was established that six additional amino acids reside at the carboxyl terminus of ORF 2 of both the Burmese and Mexican strains of HEV which do not form part of the M 3-2 and B 3-2 antigenic peptides. Since the carboxyl ends of ORF 2 and ORF 3 have been shown to be of value for the Burmese and Mexican strains of HEV, synthetic peptides corresponding to the these regions of the genome were generated for the US-1 strain of HEV. The synthetic peptides corresponding to the 48 amino acids at the carboxyl end of the ORF 2 were generated for the Burmese and Mexican strains of HEV (SEQ ID NOS:172 and 170, respectively), and are referred to as B 3-2e and M 3-2e (where "e" designates extended amino acid sequence). In addition, synthetic peptides representing the 33 amino acids at the carboxyl end of the HEV US-1 ORF 3 were generated for the Burmese and Mexican strains of HEV (SEQ ID NOS:171 and 169, respectively), and are referred to as B4-2 and M4-2. The synthetic peptide based on the epitope from within ORF 2 for the HEV US-1 strain (SEQ ID NO:174) is referred to as the US 3-2e. The synthetic peptide based on the epitope at the carboxyl end of the HEV US-1 ORF 3 (SEQ ID NO:173) is referred to as US 4-2. Each of these peptides derived from the Mexican, Burmese and US strains of HEV were synthesized, coated on a solid phase and utilized in ELISA tests to determine the relative usefulness of these synthetic peptides.

As noted in Table 42, the amino acid identity between HEV US-1 and the Burmese, Mexican, and Pakistani strains of HEV range from about 87.5% to about 91.7% for the amino acids comprising the 3-2e epitopes within ORF 2, and from about 63.6 to about 72.7% for the amino acids comprising the 4-2 epitopes within ORF 3. Without wishing to be bound by theory, given the degree of variability in the regions encoding for epitopes, it is likely that there may be strain specific antibody responses to theses viruses.

TABLE 42 – (Similarity/Identify)

	3-2e Peptide			4-2 Peptide		
	Pak	Mex	US-1	Pak	Mex	US-1
Bur	100 / 97.9	91.7 / 91.7	93.7 / 91.7	100 / 100	72.7 / 72.7	72.7 / 72.7
Pak		91.7 / 91.7	93.7 / 91.7		72.7 / 72.7	72.7 / 72.7
Mex			89.6 / 87.5			63.6 / 63.6

B. Use of ELISA's in diagnosing acute HEV infection

It has been reported that most cases of acute HEV infection in man are accompanied by IgM class antibodies which bind to one or more HEV recombinant proteins or synthetic peptides. If a person does not have IgM class antibodies to HEV, the basis for diagnosis of acute HEV infection cannot be made on serology alone but may require, RT-PCR and/or other tests to verify HEV as the etiologic agent.

C. Generation of Synthetic Peptides

Peptides were prepared on a Rainin Symphony Multiple Peptide Synthesizer using standard FMOC solid phase peptide synthesis on a 0.025 μmole scale with (HBTU) coupling chemistry by in situ activation provided by N-methyl-morpholine, with 45 minute coupling times at each residue, and double coupling at predetermined residues. Standard cleavage of the resin provided the unprotected peptide, followed by ether precipitation and washing. The peptides synthesized are shown in Table 43.

TABLE 43

Peptide	Sequence	SEQ ID NO:
B 3-2e	TLDYPARAHTFDDFCPECRPLGLQGCAFQSTVAELQRLKMKVGKTREL	SEQ ID NO:172
B 4-2	ANPPDHSAPLGVTRPSAPPLPHVVDLPQLGPRR	SEQ ID NO:171
M 3-2e	TFDYPGRAHTFDDFCPECRALGLQGCAFQSTVAELQRLKVKVGKTREL	SEQ ID NO:170
M 4-2	ANQPGHLAPLGEIRPSAPPLPPVADLPQPGLRR	SEQ ID NO:169
US 3-2e	TVDYPARAHTFDDFCPECRTLGVQGCAFQSTIAEVQRLKMKVGKTREV	SEQ ID NO:174
US 4-2	DSRPAPSVPLGVTSPSAPPLPPVVDLPQLGLRC	SEQ ID NO:173

D. Analysis of Synthesized Peptides

The synthesized peptides were analyzed for their amino acid composition as follows. The crude peptides from the small scale syntheses (0.025 μmole) were analyzed for their quality by C18 reverse phase high pressure liquid chromatography using an acetonitrile/water gradient with 0.1% (v/v) 2 trifluoracetic acid (TFA) in each solvent. From the analytical chromatogram, the major peak from each synthesis was collected and the effluent analyzed by mass spectrometry (electrospray and/or laser desorption mass spectrometry. Purification of the peptides (small and/or large scale) was achieved using C18 reverse phase HPLC with an acetonitrile/water gradient with 0.1% TFA in each solvent. The major peak was collected, and lyophilized until use.

E. ELISA Test

The utility of the HEV US-1 epitopes was determined by coating 1/4 inch polystyrene beads with each peptide. Specifically, the peptides were solubilized in water or water plus glacial acetic acid and diluted to contain 10 μg/mL in phosphate buffer (pH 7.4). A total of 60 polystyrene beads were added to a scintillation vial along with 14 mL of peptide solution (10 μg/mL) and incubated at 56°C for two hours phosphate buffered saline (PBS). After incubation, the liquid was aspirated and replaced with a buffer containing 0.1% Triton-X100®. The beads were exposed to this solution for 60 minutes, the fluid aspirated and the beads washed twice with PBS buffer. The beads then were incubated with 5% bovine serum albumin solution for 60 minutes at 40°C. After incubation, the fluid was aspirated and the beads rinsed

with PBS. The resulting beads were soaked in PBS containing 5% sucrose for 30 minutes. The fluids then were aspirated and the beads air-dried.

In one study, one-quarter inch polystyrene beads were coated with various concentrations of the synthetic peptide (approximately 50 beads per lot) and evaluated in an ELISA test (described below) using serum from an anti-HEV seronegative human as a negative control and convalescent sera from an HEV-infected person as a positive control. The bead coating conditions providing the highest ratio of positive control signal to negative control signal were selected for scaling up the bead coating process. Two 1,000 bead lots were produced for both HEV US-1 ORF 2 and ORF 3 epitopes and then used as follows.

A sample of sera or plasma was diluted in specimen diluent and mixed with antigen-coated solid phase under conditions that permit an antibody in the sample to bind to the immobilized antigen. After washing, the resulting beads were mixed with horseradish peroxidase (HRPO)-labeled anti-human antibodies that bind to either tamarin or human antibodies bound to the solid phase. Specimens which produced signals above a cutoff value were considered reactive.

More specifically, the preferred ELISA format requires contacting the antigen-coated solid phase with serum pre-diluted with specimen diluent (buffered solution containing animal sera and non-ionic detergents). Specifically, 10 µL of serum was diluted in 150 µL of specimen diluent and vortexed. Then 10 µl of this pre-diluted specimen was added to each well of an ELISA plate, followed by the addition of 200 µL of specimen diluent and an antigen coated polystyrene beads. The ELISA plate then was incubated in a Dynamic Incubator (Abbott Laboratories) with constant agitation at room temperature for 1 hour. After the incubation, the fluids were aspirated, and the wells washed three times in distilled water (5 mL per wash). Next, 200 µL of HRPO-labeled goat anti-human immunoglobulin diluted in a conjugate diluent (buffered solution containing animal sera and non-ionic detergents) was added to each well and the ELISA plate incubated for 1 hour, as indicated above. The wells then were washed three times in distilled water, the beads containing antigen and bound immunoglobulins removed from each well, and then placed in a test tube with 300 µL of a solution of 0.1M citrate buffer (pH 5.5), 0.3% o-phenylenediamine-2 HCl and 0.02% hydrogen

peroxide. After 30 minutes at room temperature, the reaction was terminated by the addition of 1 N sulphuric acid. The resulting absorbance at 492 nm was the recorded. The intensity of the color produced was directly proportional to the amount of antibody present in the test sample. For each group of specimens, a preliminary cutoff value was set to separate specimens which presumably contained antibodies to the HEV epitope from those specimens which did not.

Panel 1: Testing of pre-screened panels

In order to demonstrate the utility of epitopes derived from the HEV US-1 strain, a panel of specimens was tested by an ELISA based on the HEV US-1 amino acid sequences (Table 44 These samples had been pre-screened for antibodies to HEV, using a combination of existing peptides and a licensed anti-HEV (Abbott Laboratories) as described above and in published reports (Dawson *et al.* (1993) *supra*; Paul *et al.* (1993) *supra*).

The first 10 members of the panel consisted of specimens obtained from US volunteer blood donors whose sera was negative for antibodies to HEV following analysis using a combination of peptides and recombinant proteins derived from Burmese and Mexican strains of HEV. All the specimens were non-reactive with ELISA's derived from HEV US-1. Five additional specimens were obtained from individuals suffering from acute hepatitis, and who were diagnosed with acute HEV infection because their sera was reactive for both IgG and IgM class antibodies to HEV recombinant antigens and synthetic peptides based on the Burmese and Mexican strains of HEV. Three of the five samples were from Egypt, one from India and one from Norway (a traveler). HEV RNA was detected by RT-PCR in all five of these individuals. These five members were tested for antibodies to the HEV US-1 isolate and both IgG and IgM class antibodies were detected in each of the cases (Table 44). Thus, these data support the use of synthetic peptides from the US-1 strain of HEV as having utility in diagnosing exposure to HEV and for diagnosing acute HEV infections.

TABLE 44

Test Specimens	Licensed anti HEV		US Isolate			
			IgG		IgM	
Tested	IgG	IgM	4-2	3-2e	4-2	3-2e
Neg. Control	0.061	0.084	0.031	0.041	0.071	0.109
Pos. Control	0.567	1.051	1.606	1.619	1.376	1.798
US Volunteer Donors						
TG 827	-	-	-	-	-	-
EG 549	-	-	-	-	-	-
EC 760	-	-	-	-	-	-
RF 762	-	-	-	-	-	-
RF 762	-	-	-	-	-	-
RG 730	-	-	-	-	-	-
NH 770	-	-	-	-	-	-
AS 705	-	-	-	-	-	-
BW 494	-	-	-	-	-	-
CD 648	-	-	-	-	-	-
Egypt						
7	+	+	+	+	+	+
9	+	+	+	+	+	+
12	+	+	+	-	+	+
India	+	+	+	+	+	+
543						
Norway						
M1	+	+	+	+	+	+

Panel 2: Detection of antibodies to HEV in biological source of HEV US-1 isolate

Serial bleeds were obtained form the patient described in Example 1, whose serum served as the biological source for the HEV US-1 strain. Based on serological data obtained for the Burmese and Mexican strains of HEV, this patient would have been misdiagnosed as HEV

negative because of the lack of detectable IgM class antibodies to HEV. However, both IgM class (Table 45) and IgG class (Table 46) antibodies to the HEV US-1 strain were detected on all four bleed dates (Tables 45 and 46. Had this patient's sera been analyzed for the presence of IgG and IgM class antibodies to the HEV US 3-2e and US 4-2 peptides, a positive diagnosis of acute HEV infection would have been made. This diagnosis is further supported by the observation that the individual had acute hepatitis and most importantly, had detectable HEV US-1 strain RNA in serum samples. These data indicate that synthetic peptides derived form the HEV US-1 strain may be useful in more accurately diagnosing acute infection due to HEV.

TABLE 45

Specimens	IgM: ORF 3 synthetic peptide 4-2 ISOLATES			IgM: ORF 2 synthetic peptide 3-2e ISOLATES		
Tested	Burmese	Mexican	US-1	Burmese	Mexican	US-1
Negative Control	0.059	0.081	0.031	0.142	0.065	0.109
Positive Control	0.854	0.985	1.363	1.309	0.579	1.798
USP-1						
8 days post admission	-	-	+	-	-	+
9 days post admission	-	-	+	-	-	+
10 days post admission	-	-	+	-	-	+
37 days post admission	-	-	+	-	-	+

TABLE 46

Specimens	IgG: ORF 3 synthetic peptide 4-2 ISOLATES			IgG: ORF 2 synthetic peptide 3-2e ISOLATES		
Tested	Burmese	Mexican	US-1	Burmese	Mexican	US-1
Negative Control	0.039	0.055	0.031	0.034	0.057	0.041
Positive Control	1.296	0.666	0.941	1.322	0.893	1.041
USP-1	-	-	+	-	-	+
8 days post admission	-	-	+	-	-	+
9 days post admission	-	-	+	-	-	+
10 days post admission	-	-	+	-	-	+
37 days post admission	-	-	+	-	-	+

Panel 3 - Other cases of potential acute HEV infection

A panel of sera from 50 patients diagnosed with acute hepatitis who were negative for IgM class antibodies to the Burmese and Mexican strains was assembled. Ten of 50 sera samples were positive for antibodies to the US strain of HEV (Tables 47 and 48). RT-PCR was performed on these samples, but none of the 10 were positive for HEV RNA. Thus, as demonstrated in this example, when patient sera is analyzed for the presence of antibodies to HEV US-1, occult viral hepatitis may be diagnosed as acute HEV infection.

TABLE 47

Specimens Tested	IgM: ORF 3 synthetic peptide 4-2 ISOLATES			IgM: ORF 2 synthetic peptide 3-2e ISOLATES		
	Burmese	Mexican	US-1	Burmese	Mexican	US-1
Negative Control	0.059	0.081	0.031	0.142	0.065	0.109
Positive Control	0.854	0.985	1.363	1.309	0.579	1.798
US	-	-	-	-	-	+
Acute non A-E	-	-	-	-	-	+
SH 755	-	-	-	-	-	+
DT 314	-	-	-	-	-	+
EH 673	-	-	-	-	-	+
SG560	-	-	-	-	-	+
SR681	-	-	-	-	-	-
N11C10	-	-	+	-	-	+
35	-	-	+	-	-	+
52	-	-	-	-	-	+
161	-	-	-	-	-	+
175						

TABLE 48

Specimens Tested	IgG: ORF 3 synthetic peptide 4-2 ISOLATES			IgG: ORF 2 synthetic peptide 3-2e ISOLATES		
	Burmese	Mexican	US-1	Burmese	Mexican	US-1
Negative Control	0.039	0.055	0.031	0.034	0.057	0.041
Positive Control	1.296	0.666	0.941	1.322	0.893	1.041
US	-	-	-	-	-	-
Acute non A-E	-	-	-	-	-	-
SH 755	-	-	-	-	-	-
DT 314	-	-	-	-	-	-
EH 673	-	-	-	-	-	-
SG560	-	-	-	-	-	-
SR681	-	-	-	-	-	+
N11C10	-	-	-	-	-	-
35	-	-	-	-	-	+
52	-	-	-	-	-	-
161	-	-	-	-	-	-
175						

Example 9 - Animal Transmission Studies

Cynomolgus macaques (*Macaca fascicularis*) were obtained through the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, Texas. The animals were maintained and monitored in accordance with guidelines established by SFBR to ensure humane care and the ethical use of primates. Sera were obtained twice weekly for at least four weeks prior to inoculation in order to establish the baseline levels for serum ALT. Cut-off (CO) values were determined based on the mean of the baseline plus 3.75 times the standard deviation. Two macaques were inoculated intravenously with 0.4-0.625 mL of HEV positive USP-1 serum and one macaque was inoculated with 2.0 mL of HEV positive USP-2 serum. Serum and fecal samples were collected twice weekly for up to 16 weeks post-inoculation (PI). Sera were tested for changes in ALT and values greater than the CO were considered positive and suggestive of liver damage. Sera samples were tested for antibodies to HEV as described

hereinabove in Example 8 (Table 49, Figure 7). Sera and fecal samples were tested for HEV RNA by RT-PCR. 25-100 µL of macaque sera was extracted using the QIAamp Viral RNA Kit (Qiagen). 10% fecal suspension were extracted as described in Example 1. RT PCR was performed as described below in Example 12 (Figure 7).

Although intravenous inoculation of 0.4-0.625 mL of USP-1 sera into two cynomolgus macaques failed to produce infection (data not shown), inoculation of 2.0 mL of sera from patient US-2 resulted in viremia and elevations of liver enzyme levels in the serum (Figure 7). HEV RNA was first detected in fecal material on day 15 PI and remained positive through 64 days PI. Serum specimens collected between days 28-56 PI were HEV RNA positive. Elevated ALT values were noted on days 15, 44-58, 72 and 93 PI, with the peak ALT value (116 IU/L) on day 51 PI.

Six ELSIAs based on the Burmese, Mexican and US sequences for the 4-2 and 302e peptides were utilized to assess antibody response. Measurable response was found only to the US 3-2e peptide assay (Table 49) with no noted crossreactivity to the Burmese or Mexican peptides. IgM class antibody directed against HEV was detectable between 28 and 58 days PI. This was followed by a strong anti-HEV-IgG response at day 44 PI.

TABLE 49

Date	DPI	ALT	AST	GGT	IgG S/N
06/04/97	-82	35	37	102	1.4
06/06/97	-80	39	32	90	
06/11/97	-75	38	36	100	
06/13/97	-73	36	46	86	
06/18/97	-68	45	30	85	
06/20/97	-66	43	37	87	
06/25/97	-61	37	30	92	
06/27/97	-59	42	36	87	
08/25/97	0	41	36	107	1
08/27/97	2				
09/02/97	8	34	34	102	
09/04/97	10	34	31	91	
09/09/97	15	58	42	108	0.8
09/10/97	16	44	45	93	
09/15/97	21	35	32	86	
09/17/97	23	49	71	88	
09/22/97	28	39	33	86	1.2
09/24/97	30	40	37	90	
09/29/97	35	41	40	80	
10/01/97	37	48	58	90	1.1
10/03/97	39				
10/06/97	42	45	33	89	
10/08/97	44	58	38	94	6.2
10/15/97	51	116	62	89	11.9
10/20/97	56	87	38	83	33.6
10/22/97	58	76	43	85	29.9
10/28/97	64	45	42	88	17.2
10/29/97	65	46	34	88	
11/03/97	70	39	54	85	
11/05/97	72	54	47	88	13.3
11/10/97	77	47	33	93	
11/12/97	79	50	38	93	12.4
11/17/97	84	46	31	91	10.4
11/19/97	86	52	41	88	
11/26/97	93	67	104	109	7.2
12/03/97	100	36	36	108	
12/09/97	106	38	34	115	
12/10/97	107	36	29	103	2.1

Example 10: Recombinant Protein ELISAs

A. Recombinant Constructs

E. coli derived recombinant proteins encoded by HEV-US sequence from the ORF 2 and ORF 3 regions of the HEV-US genome were expressed as fusion proteins with CMP-KDO synthetase (CKS), designated as pJOorf3-29 (SEQ ID NO:191); cksorf2m-2 (SEQ ID NO:192); and CKSORF32M-3 (SEQ ID NO:193), or as non-fusion proteins, designated as plorf3-12 (SEQ ID NO:194); plorf2-2.6 (SEQ ID NO:195); and PLORF-32M-14-5 (SEQ ID NO:196). The cloning vector pJO201, as described in U.S. Patent No. 5,124,255, was used in the construction of the recombinant fusion proteins. This vector was digested with the restriction endonucleases *Eco RI* and *Bam HI* to allow cloning of HEV-US sequences in frame with CKS. The lambda pL expression vector pKRR826 was utilized in the construction of recombinant non-fusion proteins. This vector was digested with the restriction endonucleases *Eco RI* and *Bam HI* to allow for cloning of HEV-US sequences immediately down stream of the ribosome binding site. Since the vector system contains strong lambda promoter, induction of heterologous protein synthesis is accomplished by shift in the temperature from 30°C to 42°C which inactivates the temperature sensitive repressor protein. The constructs were cloned and transformed into *E. coli* K12 strain HS36 cells for the expression of these HEV proteins.

HEV-US sequences were amplified from nucleic acids extracted from HEV US-2 human serum or macaque 13906 fecal material and reverse transcribed as described above in Example 5. The ORF 2 sequence, encompassing the carboxyl half of ORF 2 (*i.e.*, encoding amino acid residue numbers 334-660 of SEQ ID NO:167), was generated using a sense primer, SEQ ID NO:208, which contained an *Eco RI* restriction site as well as an ATG start codon and an antisense primer, SEQ ID NO:198, which contained a unique peptide sequence termed FLAG (Eastman Kodak), two consecutive TAA termination codons, and a *Bam HI* restriction site. A 50 µl PCR reaction was set up using LA TAQ (Takara) reagents as recommended by the manufacturer. Cycling conditions involved 40 cycles of 94°C for 20 seconds, 55°C for 30 seconds, 72°C for 2 minute. Amplifications were preceded by 1 minute at 94°C and followed by 10 minutes at 72°C. Products were digested with *Eco RI* and *Bam HI* and ligated into the

desired vector. The nucleotide sequence of the CKS fusion clone, between the restriction sites, is set forth in SEQ ID NO:192, the translation of which is set forth in SEQ ID NO:199. The nucleotide sequence of the non-fusion clone, between restriction sites, is set forth in SEQ ID NO:195, the translation of which is set forth in SEQ ID NO:200. The ORF 3 sequences, encompassing the entire ORF 3 (amino acids 1-122), was generated using a sense primer, SEQ ID NO:201, which contained an *Eco RI* restriction site as well as an ATG start codon and an antisense primer, SEQ ID NO:202, which contained a unique peptide sequence termed FLAG, two consecutive TAA termination codons, and a *Bam HI* restriction site. A 50 μL PCR reaction was set up using Qiagen reagents as described in Example 5. Cycling conditions comprised 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute. Amplifications were preceded by incubation for 1 minute at 94°C, followed by 10 minutes at 72°C. The resulting products were digested with *Eco RI* and *Bam HI* and ligated into the desired vector. The nucleotide sequence of the CKS fusion clone, between the restriction sites, is set forth in SEQ ID NO:191, the translation of which is set forth in SEQ ID NO:203. The nucleotide sequence of the clone representing the non-fusion construct, between the restriction sites, is set forth in SEQ ID NO:195, the translation of which is set forth in SEQ ID NO:204.

Additionally, a chimeric construct encompassing the full length ORF 3 (amino acids 1-123) and the carboxyl half of ORF 2 (amino acids 334-660) was generated. Approximately 100 ng of the plasmids containing SEQ ID NO:191 and SEQ ID NO:192 were utilized as template in 100 μL PCR reactions. PCR buffers and enzymes were from the LA TAQ kit (Takara), and used in accordance with the manufacturer's instructions. ORF 3 was amplified with primers set forth in SEQ ID NOS:201 and 205. The antisense primer of SEQ ID NO:205 eliminates the FLAG sequences and stop codons from the carboxyl end of SEQ ID NO:191 and contains the sequence identical to SEQ ID NO:192 which will eliminate the ATG start codon. ORF 2 was amplified with primers of SEQ ID NOS:208 and 198. Cycling conditions were as described above using LA TAQ. The resulting products were fractionated on a 1.2% agarose gel and excised. DNA was isolated from the gel slices using GeneClean II as described by the manufacturer (Bio101). Products were eluted off the glass beads into 15 μL H_2O. Approximately equal molar ratios of each product (10 μL of ORF 3 product and 1 μL of ORF 2 product) were mixed in a 25 μL end fill reaction using 1x PCR buffer, 0.5 μl dNTPs, and 0.25

μL LA TAQ (Takara). This reaction was cycled as follows: 94°C for 1 minute, 10 cycles of 94°C for 20 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes, followed by 72°C for 10 minutes. 5 μL of this reaction was placed into a 100 μL amplification reaction utilizing LA TAQ kit (Takara) and primers of SEQ ID NOS:201 and 198. Cycling conditions were 94°C for 1 minute followed by 35 cycles of 90°C for 20 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes. This was followed by 10 minutes at 72°C and a 4°C soak. Products of the appropriate size were digested with restriction enzymes *Eco RI* and *Bam HI*. This product was ligated into pJO201 and clones with the appropriate sequence identified (SEQ ID NO:193, the translation of which is set forth in SEQ ID NO:206). The resulting product was ligated into pKRR826 and clones with the appropriate sequence (SEQ ID NO:196, the translation of which is set forth in SEQ ID NO:207) identified.

B. Protein expression and purification

The CKS constructs were expressed in two 500 mL cultures (4 hour induction), as described in U. S. Patent No. 5,312,737. P_L constructs were expressed as described above. Frozen cell pellets of the induced *E.coli* cultures were used as the starting material for the purification of protein. Cells were lysed in buffer containing lysozyme, DNase and proteinase inhibitors. Soluble protein was separated from insoluble (inclusion body) protein by centrifugation at 11,000 x g. The solubility of the recombinant protein was estimated via sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and Western blotting using a FLAG® M2 antibody.

Soluble recombinant protein was purified by affinity chromatography using FLAG® M2 antibody affinity gel after exchange into suitable buffer (Surowy *et al.* (1997) Journal of General Virology, 78:1851-1859). If necessary, additional purification was performed via Sephacryl® S-200 gel filtration chromatography, in which the sample and chromatography buffers contained 10 mM β-mercaptoethanol. Purified protein was quantitated by measurement of absorbance at 280 nm. An assumed extinction coefficient of 1 was used to convert absorbance to mg of protein. Protein purity was determined by scanning densitometry

(Molecular Dynamics) of protein fractioned by SDS PAGE, using standards of pre-determined purity.

C. ELISA

In order to determine potential utility of the recombinant HEV US constructs, solid phase ELISA's were developed and evaluated. All recombinant HEV US proteins were coated onto solid phase as described below. Briefly, 1/4" polystyrene beads were coated with varying amounts of (PJOORF3-29) which ranged in concentration from 0.5 to 10 μg/mL diluted in 100 mM sodium phosphate buffer, pH 7.6. Sixty beads per concentration condition were coated in approximately 14 mL of buffer and rotated end-over-end at 40° C for 2 hours. The coating solution was aspirated and the remainder of the coating procedure was performed as described above in Example 8, section E, paragraph 1.

An ELISA was developed using the pJOorf3-29 coated beads. Briefly, sera or plasma was diluted 1:16 in Specimen Diluent (SpD) as described above. A 10 μL aliquot of this pre-dilution then was added into the well of a reaction tray, followed by the addition of 200 μL of SpD. One coated bead was added per well and incubated for 1 hour at 37°C in dynamic mode using a Dynamic Incubator (Abbott Laboratories). After incubation, the fluid was aspirated and each bead washed 3 times with deionized water (5 mL per wash). The beads then were incubated with 200 μL HRPO-labeled goat anti-human IgG or IgM conjugate, diluted in conjugate diluent (described above) and incubated for 30 minutes at 37°C. The conjugate then was aspirated and the beads washed as above. Color development and absorbance readings were performed as described in Example 8, section E.

To validate the immunoreactivity of this construct, serial bleed specimens from Macaque #13903 experimentally infected with HEV US-2 (described in Example 9) were tested for IgM and IgG antibody to pJOorf3-29. As shown in Figure 1, IgM antibody was detected at day 51 post-infection (PI) and continued to be elevated through day 72 and corresponded to the peak elevations in ALT values. IgG antibody to pJOorf3-29 was first detected on day 56 PI and remained positive through day 107 (Table 50).

A second construct, plorf3-12, representing HEV US ORF 3 but lacking the CKS fusion partner was also evaluated in an ELISA format identical to that described above. IgG antibody to plorf3-12was evaluated on several serial bleeds from the same experimentally infected macaque. IgG antibody to plorf3-12was detected on day 58 PI and remained positive through day 107 (Table 50).

TABLE 50

	pJOorf3-29		**plorf3-12**	
Sample	Mean OD	S/N	Mean OD	S/N
SpD			0.01	
"pre-bleed"	0.02		0.01	
Post-inoculation bleeds - Days Post-inoculation (DPI)				
DPI				
44	0.02	0.96	0.02	1.07
51	0.05	2.35	0.03	2.25
56	0.24	10.35	0.05	3.43
58	0.44	19	0.16	11.57
63	1.14	49.57	0.32	22.82
65		NT	0.53	37.54
70		NT	1.19	85.04
72	2.22	96.52	0.92	65.71
98	0.89	38.87	0.39	27.86
107	0.49	21.43	0.27	19.36
NT: not tested				

Due to the high percent homology between Swine HEV and the US-2 isolate, the pJOorf3-29 ELISA also was used to measure the prevalence of both immunoreactive IgG and IgM in sera isolated from U.S. swine herds (Table 51). The assay was performed as described above with the exception of substituting HRPO-conjugated labeled anti-swine immunoglobulin (either IgG or IgM) for the anti-human conjugate.

TABLE 51

Prevalence of Antibody to HEV orf3 in U. S. Swine (pJOorf3-29)					
Swine Source State	IgG Reactive No./Total (%)	No. IgG Confirmed by Blocking or Blot (%)	IgM Only Reactive No./Total (%)	No. IgM Only Confirmed by Blot (%)	Total Exposure Confirmed Only
New Jersey	9/14 (64)	9 (100)	0/14		64%
Texas	25/50 (50)	20 (80)	0/50		40%
Iowa	7/64 (11)	1 (14)	0/64		2%
Oregon	7/36 (19)	5 (71)	1/36 (3)	1/1 (100)	14%
Total	48/164 (29)	35 (73)	1/164 (0.6)	1/1 (100)	36/164 (22%)
NOTE: A total of 4 pigs (all Texas herd) had IgM in addition to IgG.					

In order to confirm reactive specimens, a blocking assay was developed. Briefly, a 10 μL aliquot of the 1:16 specimen pre-dilution was added to duplicate wells of a reaction tray; one well to be used for the standard assay and one well to be used for the blocking assay. The ELISA for the standard assay was performed as described above with the exception that there was a 30 minute room temperature pre-incubation step prior to addition of the pJOorf3-29 antigen coated bead. For the blocking assay, pJOorf3-29 was added to the SpD (blocking reagent) at a 10-fold molar excess to that on the solid phase. 200 μL of blocking reagent was added per reaction and a 30 minutes room temperature pre-incubation was performed prior to addition of the pJOorf3-29 antigen coated bead. The rest of the assay was performed as described above for the swine assay, except that the HRPO-conjugated anti-swine conjugate (IgG) was used in place of the anti-human conjugate.

The % blocking was determined using the equation:

$$[(A_{492\ nm}\ \text{standard assay} - A_{492\ nm}\ \text{blocking assay})/A_{492\ nm}\ \text{standard assay}] \times 100$$

Specimens that showed blocking rates of 50% or greater were considered to be reactive for IgG antibody to HEV pJOorf3-29. Representative IgG positive and IgG negative swine samples and their blocking results are shown in Table 52.

Table 52 - Blocking Assay With pJOorf3-29 and PL-12 at 10-fold molar excess

	Standard Assay			Blocking Assay w/ pJOorf3-29 at 10-fold molar excess			BLOCKING RESULT
	SAMPLE	OD	MEAN OD	OD	MEAN OD	% BLOCKING	
	NC	0.02 0.02 1.09	0.02	0.02 0.03 0.56	0.02		
	PC	1.01	1.05	0.48	0.52	50.4%	+
	Oregon Swine Panel Positives						
1	NJ5	0.65		0.15		76.5%	+
2	NJ12	1.78		0.46		74.0%	+
3	NJ21	0.48		0.16		66.7%	+
4	NJ23	0.52		0.09		81.9%	+
5	T5	2		0.81		59.5%	+
6	T9	0.52		0.18		64.3%	+
7	T32	2		0.9		54.9%	+
8	T33	0.3		0.13		57.8%	+
9	T48	0.53		0.14		73.7%	+
10	T49	0.33		0.09		73.3%	+
	Oregon Swine Panel Negatives						
11	T43	0.08		0.07		13.3%	-
12	T46	0.12		0.08		29.1%	-
13	I-23	0.12		0.08		32.2%	-
14	I-24	0.07		0.06		13.2%	-
15	I-27	0.1		0.08		12.6%	-
16	I-28	0.15		0.12		20.4%	-
17	I-33	0.15		0.12		19.9%	-
18	I-39	0.23		0.14		37.4%	-
19	I-61	0.19		0.14		25.9%	-
20	O-4	0.15		0.12		22.7%	-

In addition to the blocking assay, western blots were run on a subset of swine specimens. Briefly, 50 μg of HEV pJOorf3-29 and 50 μg of "CKS only" proteins were fractionated by SDS-PAGE and the fractionated proteins transferred to nitrocellulose. 3mm strips of the nitrocellulose were cut and incubated overnight at room temperature on an orbital rotator with primary antibody at a 1:100 dilution in protein based buffer containing 10% *E. coli* lysate. On the following day, strips were washed three times with 0.3% Tween/TBS (TBST), followed by the addition of HRPO-conjugated anti-swine IgG conjugate diluted to 0.5 μg/mL in TBST. Strips were incubated with rotation for 4 hours at room temperature. Blots then were washed three times in TBST, followed by 2 washes in TBS. Blots were developed using 4-chloro-1-naphthol as a substrate. The reaction was stopped by the addition of water and band intensities recorded. Specimens were determined to have specific reactivity to HEV if they showed a band at the correct molecular weight for pJOorf3-29 (approx. 40 kD) and had no reactivity in the region where "CKS only" bands (approx. 29 kD). Results for 20 swine sera run on the pJOorf3-29 western blot are shown in Table 53. No swine sera showed non-specific reactivity with the "CKS-only" band.

TABLE 53

	BAND INTENSITY	
Swine ID Number	pJOorf3-29	CKS only
NJ4	+	-
NJ7	+	-
NJ14	+++	-
NJ18	+	-
NJ25	++++	-
T6	++++	-
T10	++++	-
T14	-	-
T15	+	-
T18	++	-
T28	+++	-
T29	-	-
T30	+	-
T34	-	-
T36	++++	-
T37	-	-
T43	-	-
T44	++++	-
T45	++++	-
T46	-	-

These data suggest that HEV US recombinant proteins are useful in diagnosing exposure to HEV.

Example 11 - Consensus Primers

Consensus oligonucleotide primers for HEV ORF 1 ORF 2 and ORF 3 were designed based on conserved regions between the full length sequences of isolates from Asia, Mexico, and the US (Figure 9). The ORF 1 primers are positioned within the methyltransferase region at nucleotides 56-79 and 473-451 of the Burmese isolate (GenBank accession number M73218), and amplify a product 418 nucleotides in length. The ORF 1 primers include:

HEVConsORF 1-s1; CTGGCATYACTACTGCYATTGAGC (SEQ ID NO:147); and HEVConsORF 1-a1; CCATCRARRCAGTAAGTGCGGTC (SEQ ID NO:148).

The ORF 2 primers, at positions 6298-6321 and 6494-6470 of the Burmese isolate, produce a product 197 nucleotides in length. The ORF 2 primers include:

HEVConsORF 2-s1; GACAGAATTRATTTCGTCGGCTGG (SEQ ID NO:150); and
HEVConsORF 2-a1; CTTGTTCRTGYTGGTTRTCATAATC (SEQ ID NO:126).

For a second round of amplification, internal primers can be used to produce products 287 and 145 nucleotides in length for ORF 1 and ORF 2, respectively. The ORF 1 primers include:

HEVConsORF 1-s2; CTGCCYTKGCGAATGCTGTGG (SEQ ID NO:177); and
HEVConsORF 1-a2; GGCAGWRTACCARCGCTGAACATC (SEQ ID NO:178).

The ORF 2 primers include:

HEVConsORF 2-s2; GTYGTCTCRGCCAATGGCGAGC (SEQ ID NO:152); and
HEVConsORF 2-a2; GTTCRTGYTGGTTRTCATAATCCTG (SEQ ID NO:128).

PCR reactions contained 2 mM $MgCl_2$ and 0.5 μM of each oligonucleotide primer as per the manufacturer's instructions (Perkin-Elmer) and amplified using Touch-down PCR as described in Example 5. Amplified products were separated on a 1.5% agarose gel and analyzed for the presence of PCR products of the appropriate size. The primers were used to detect the presence of virus in serum and feces containing HEV US-2 as described above in Example 8 and Figure 7. In addition, these primers were found to be reactive with a number of different variants of HEV that included Burmese-like strains 6A, 7A, 9A and 12 A as well as two distinct isolates from Greece (see Example 13 below) as well as a unique isolate from Italy and the two isolates from the US (see Example 13 below). In addition, these primers have been used to identify an isolate from a patient with a clinical diagnosis of acute sporadic hepatitis from the Liaoning province of China (S15). The results are presented in Table 54 below.

TABLE 54

Sample	ORF 1 -PCR1	ORF 1 -PCR 2	ORF 2 - PCR1	ORF 2 -PCR2
6A	neg	pos	pos	Pos
7A	neg	pos	neg	Pos
9A	neg	neg	neg	Pos
12A	pos	pos	neg	Neg
G1	pos	pos	pos	Pos
G2	pos	pos	pos	Pos
Itl	pos	pos	pos	Pos
S15	nd	pos	nd	Pos
US-2	pos	pos	pos	Pos

Example 12 - Detection of HEV RNA in Primary Human Fetal Kidney Cells

Frozen cell pellets containing $10x10^6$ cells were thawed and resuspended in 1.0 mL Dulbecco's phosphate buffered saline. RNA was extracted from 20 µL ($2x10^5$ cells) of the cell pellet using the Ultraspec Isolation System as described in Example 1. cDNA synthesis was performed on the above extracted nucleic acid (RNA) and primed with random hexamers. PCR then was performed on the above cDNA using degenerate primers from the ORF-1 and ORF-2 regions of the viral genome at a final concentration of 0.5µM as described in Example 11.

To monitor the performance of the above assay, a positive control utilizing primary human kidney cells and HEV US-2 positive serum was included in the experimental design. Two positive control sets were prepared by spiking $2x10^5$ HEV negative primary human kidney cells with 2.5 µL and 25 µL of a documented HEV US-2 positive serum specimen. The positive control serum also was tested without the addition of the human kidney cells.

Nineteen primary human kidney cell pellet lots were tested using the above assay method utilizing the 2 degenerate primer sets from ORF 1 and ORF 2. The results are summarized in Table 55 below. None of the cell pellet lots tested gave positive results as seen in the positive controls.

TABLE 55

CELL LINES	PCR RESULTS
1757	-
1851	-
1690	-
1853	-
1906	-
1935	-
1838	-
1955	-
1893	-
1895	-
1699	-
1877	-
1942	-
1844	-
1840	-
1875	-
1921	-
1946	-
1846	-
cells + 25 µL serum	+
cells + 2.5 µL serum	+
25 µL serum	+

Example 13: Identification and Extension of Additional US-type Isolates

A. Identification of isolate from Italy, referred to as It1

RNA was extracted from 25 to 50 µL of serum using the QIAamp Viral RNA kit (Qiagen) as described by the manufacturer except that 25 to 50µL of serum was diluted to 100µL with PBS and the final elution was performed with 100 µL of RNase-free water. RT reactions were random primed. PCR utilized the HEV US-1 primer as described hereinabove in Example 5. A 294 bp product was generated after amplification with primers SEQ ID NO:94

and SEQ ID NO:96. The product was cloned and sequenced as described in Example 3 and is shown in SEQ ID NO:179.

Extension of the Itl isolate genome was performed as follows. RNA was extracted from 25 to 50 μL of serum as described hereinabove in Example 5. RT reactions were random primed. PCR utilized the HEV CONSENSUS primers described above in Example 11 using touchdown PCR, as described hereinabove in Example 3. Primers shown in SEQ ID NOS:147 and 148 were used to generate a product having the sequence set forth in SEQ ID NO:180 (reaction z2, 418 bp). Primers as shown in SEQ ID NOS:150 and 126 were used to generate a product having the sequence set forth in SEQ ID NO:181 (reaction z3, 197 bp). In the presence of 1x PCR Buffer and 20% Q Solution (Qiagen), primers as shown in SEQ ID NOS:182 and 183 were used to generate a product having a sequence set forth in SEQ ID NO:184 (reaction z4, 234 bp). The 3' end of the genome was isolated by 3' RACE as described above in Example 3 using primers shown in SEQ ID NOS:150 and 85 in PCR1, and primers shown in SEQ ID NOS:152 and 85 in PCR2, to produce a product having the sequence shown in SEQ ID NO:185 (reaction z5, 890 bp). Products were cloned and sequenced as described in Example 3 and consensus sequences generated. These regions are shown in Figure 8 and are set forth in SEQ ID NOS:180, 184 and 186. The amino acid translations of these regions are represented by the amino acid sequences set forth in SEQ ID NOS:187, 188; 189; 190; and 197.

B. Identification of two isolates from Greece, referred to as G1 and G2

Two patients with acute hepatitis who had no history of travel to endemic areas had been analyzed with primers based on the Burmese isolate (Psichogiou M.A., *et al.*, (1995) "Hepatitis E virus (HEV) infection in a cohort of patients with acute non-A, non-B hepatitis," Journal of Hepatology, 23, 668-673). Only patient G2 was found to be PCR positive. RNA was isolated as described hereinabove in Example 12 and PCR performed with the consensus primers described above in Example 11. The ORF 1 and ORF 2 primer sets generated products of the expected size from both patients. The products were cloned and sequenced as described above in Example 3. The products generated using the ORF 1 and ORF 2 consensus primers from patient G1 are shown in SEQ ID NOS:209 and 211, respectively. The products generated

using the ORF 1 and ORF 2 consensus primers from patient G2 are shown in SEQ ID NOS:213 and 215, respectively. The identification of G1 as being PCR positive demonstrates the utility of the consensus primers over Burmese base strain specific primers.

Additional sequence from G1 and G2 was also obtained using primers SEQ ID NO:16, SEQ ID No:17, and SEQ ID NO:18 as for the generation of SEQ ID NO:19 as described above in Example 3 except that random primed cDNA was used for PCR and amplification involved 10 cycles of 94°C for 20 seconds, 60°C for 30 seconds, and 72°C for 1 minute, followed by 10 cycles of 94°C for 20 seconds, 55°C for 30 seconds, and 72°C for 1 minute followed by 30 cycles of 94°C for 20 seconds, 50°C for 30 seconds (-0.3°C/cycle), and 72°C for 1 minute. This was followed by an extension cycle of 72°C for 7 minutes. The product generated from patient G1 is shown in SEQ ID NO:217. The product generated from patient G2 is shown in SEQ ID NO:220.

Alignments of the nucleotide sequences of the US, Chinese, Greek, Italian, Mexican and Burmese-like isolates, were performed to determine the relationship of these isolates to each other. The divergence of the Italian isolate is supported by the comparisons of the product from the ORF 1 region of the genome which has a percent nucleic acid identity of 77.6 %, 78.4 %, and 84.6 % with the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), respectively (Table 36). The divergence of the Italian isolate also is supported by the comparisons of the product from the ORF 2 region of the genome which had a percent nucleic acid identity of 83.3 %, 79.7 %, and 87.8 % with the prototype isolates from Burma, Mexico and the US, respectively (Table 37). The nucleotide identities between the prototype isolates from Burma, Mexico and the US, range between 75.5 % to 82.4 % over these two regions. Over these same regions, the isolates that comprise the Burmese-like group have much higher identities of 91.2% or greater.

Comparisons of the ORF 1 and ORF 2 amplified sequences indicate that the isolates from the two patients from Greece are quite distinct from each other, exhibiting 84.4 % and 87.2 % nucleotide sequence identity over these regions of ORF 1 and ORF 2, respectively. At the nucleotide level, the percent identities between the Greek, Italian and US isolates range from 81.9% to 86.8% for the ORF 1 product (Table 36) and 82.4% to 87.8% for the ORF 2 product

(Table 37). These values are lower than the lowest percent nucleotide identities between any Burmese-like isolates, which are greater than 91.2% for both ORF 1 and ORF 2. Comparisons of the amino acid identities derived from the ORF 1 fragment between the US, Italian or Greek isolates and the Burmese or Mexican isolates range from 87.8% to 93.5 % (Table 36). These values are equal to or less than the differences between the Burmese and Mexican isolates (93.5% to 95.1 %) (Table 36), indicating that the isolates from non-endemic regions are distinct from the isolates originating from endemic regions.

The relative evolutionary distances between the viral sequences analyzed are readily apparent upon inspection of the unrooted phylogenetic trees generated from the pairwise distances, where the branch lengths are proportional to the relative genetic relationships between the isolates. The phylogenetic trees based on alignments of either ORF 1 (Fig. 10) or ORF 2 (Fig. 11) sequences are quite similar in overall topology. The Burmese-like isolates and the Mexican isolate represent major branches at one end of the tree. The human US isolates form a distinct group distal to the Mexican and Burmese isolates. The swine HEV-like sequence from ORF 2 is closely related to the US human isolates. The three European isolates form three additional distinct branches with the Italian isolate being most closely related to the US isolates.

Example 14: Identification Additional US-type Isolates from Austria and Argentina

RNA was isolated from serum from three patients with acute hepatitis who had no history of travel to areas considered endemic for HEV as described hereinabove in Example 12 and PCR performed with the consensus primers described above in Example 11. One patient was from Austria, Au1, (Worm, *et al.*, (1998) "Sporadic hepatitis E in Austria," New England Journal of Medicine, 339, 1554-1555) while the other two patients were from Argentina. The ORF 1 and ORF 2 primer sets generated products of the expected size from all patients. The products were cloned and sequenced as described above in Example 3. The products generated using the ORF 1 and ORF 2 consensus primers from patient Au1 are shown in SEQ ID NOS:243 and 245, respectively. The products generated using the ORF 1 and ORF 2 consensus primers from patient Ar1 are shown in SEQ ID NOS:247 and 249, respectively. The

products generated using the ORF 1 and ORF 2 consensus primers from patient Ar2 are shown in SEQ ID NOS:251 and 253, respectively. PCR products were obtained after both the first round of ORF1 PCR with the a1 and s1 primers as well as the second round of nested ORF1 PCR with the a2 and s2 primers for Au1, Ar1 and Ar2. PCR products were obtained after both the first round of ORF2 PCR with the a1 and s1 primers as well as the second round of nested ORF2 PCR with the a2 and s2 primers for Au1 and Ar2. Product from Ar1 was detected only after the second round of nested ORF2 PCR with the a2 and s2 primers.

Alignments of the nucleotide sequences of the US, Chinese, Greek, Italian, Austrian, Argentine, Mexican and Burmese-like isolates, were performed to determine the relationship of these isolates to each other as described in Example 6. The divergence of the Austrian isolate, Au1, is supported by the comparisons of the product from the ORF 1 region of the genome which has a percent nucleic acid identity of 77.1 %, 78.2 %, and 87.9 % with prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), respectively (Table 56). The divergence of the Austrian isolate also is supported by the comparisons of the product from the ORF 2 region of the genome which had a percent nucleic acid identity of 85.1 %, 79.1 %, and 83.1 % with the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), respectively (Table 57). The divergence of the Argentine isolate, Ar2, is supported by the comparisons of the product from the ORF 1 region of the genome which has a percent nucleic acid identity of 76.0 %, 76.0 %, and 84.9 % with the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), respectively (Table 56). The divergence of the Ar2 isolate also is supported by the comparisons of the product from the ORF 2 region of the genome which had a percent nucleic acid identity of 85.8 %, 82.4 %, and 85.8 % with the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), respectively (Table 57). The divergence of the Argentine isolate, Ar1, is supported by the comparisons of the product from the ORF 1 region of the genome which has a percent nucleic acid identity of 76.6 %, 77.6 %, and 85.7 % with the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), respectively (Table 56). The nucleotide identities between the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1), range between 75.5 % to 82.4 % over these two regions. Over these same regions, the isolates that comprise the Burmese-like group have much higher identities of 91.2% or greater. Although only a nested ORF2 PCR product was obtained from the Argentine isolate, Ar1, the

divergence of the Ar2 isolate also is supported by the comparisons of this smaller product from the ORF 2 region of the genome which had a percent nucleic acid identity of 80.6 % with the prototype isolates from Burma (B1), Mexico (M1) and the US (US-1) (Table 57).

At the nucleotide level, the percent identities between the Austrian, Argentine, Greek, Italian and US isolates (excluding the identity between US-1 and US-2) range from 80.6% to 89.8% for the ORF 1 product (Table 56). At the nucleotide level, the percent identities between the Austrian, Argentine, Greek, Italian and US isolates (excluding the identity between US-1 and US-2 and Ar-1 and Ar-2) range from 80.6% to 89.2% for the ORF 2 product (Table 57). These values are lower than the lowest percent nucleotide identities between any Burmese-like isolates, which are 91.2% or greater for ORF 1 and ORF 2.

TABLE 56

Nucleotide and deduced amino acid identity between isolates of HEV over 371 base (123 amino acid) ORF 1 fragment

Nucleotide Identity

Ar1	88.4	89.8	84.4	81.9	85.4	85.7	85.2	82.5	76.6	76.6	79.0	78.2	79.2	77.1	78.4	76.8	78.7	77.6
98.4	**Ar2**	87.9	80.6	81.1	84.4	84.9	85.4	83.3	76.0	76.6	74.9	75.7	75.7	74.1	77.4	76.8	75.7	76.0
100	98.4	**Au1**	85.2	81.1	86.0	87.9	87.1	84.6	77.1	77.6	76.8	76.6	77.6	76.6	77.4	78.2	77.1	78.2
99.1	97.6	99.1	**G1**	84.4	84.1	81.9	82.5	83.0	78.4	77.9	77.6	78.2	77.9	76.6	77.6	78.2	77.4	76.6
98.4	96.7	98.4	99.2	**G2**	81.7	83.8	83.0	81.9	78.2	77.6	78.2	77.9	78.4	77.1	77.9	77.6	78.7	76.8
97.6	95.9	97.6	96.7	96.7	**It1**	84.6	86.8	84.9	77.6	77.6	77.1	77.4	77.4	76.3	77.6	77.4	77.6	78.4
99.2	97.6	99.2	98.4	97.6	96.7	**US-1**	91.9	90.8	75.5	74.9	75.2	75.2	75.7	75.2	76.6	75.5	76.0	76.6
100	98.4	100	99.2	98.4	97.6	99.2	**US-2**	89.9	75.2	75.4	75.2	75.4	76.0	74.9	75.7	75.7	76.3	77.6
97.6	95.9	97.6	96.7	95.9	95.1	96.7	97.6	**S1**	76.6	76.6	74.7	74.9	74.4	73.6	75.2	74.9	75.2	75.7
91.1	90.2	91.1	90.2	90.2	92.7	91.9	91.1	88.6	**B1**	98.7	94.6	94.6	94.9	94.3	94.3	96.0	94.6	79.0
91.1	90.2	91.1	90.2	90.2	92.7	90.2	91.1	89.6	98.4	**B2**	93.8	93.8	94.1	93.5	93.5	95.1	93.8	78.4
89.4	88.6	89.4	88.6	88.6	91.1	90.2	89.4	87.0	98.4	96.7	**C1**	97.8	98.1	96.8	92.7	91.6	97.3	79.8
90.2	89.4	90.2	89.4	89.4	91.9	91.1	90.2	87.8	99.2	97.6	99.2	**C2**	98.7	97.6	93.8	91.6	98.4	79.5
90.2	89.4	90.2	89.4	89.4	91.9	91.1	90.2	87.8	99.2	97.6	99.2	100	**C3**	97.3	93.5	91.9	98.1	79.5
89.4	88.6	89.4	88.6	88.6	91.9	90.2	89.4	87.0	98.4	96.7	99.2	99.2	99.2	**C4**	93.0	91.9	97.0	78.7
90.2	89.4	90.2	89.4	89.4	91.9	91.1	90.2	87.8	99.2	97.6	97.6	98.4	98.4	97.6	**I1**	91.4	93.3	79.2
88.6	87.8	88.6	87.8	87.8	90.2	89.4	88.6	86.2	97.6	95.9	95.9	96.7	96.7	95.9	96.7	**I2**	91.6	78.4
90.2	89.4	90.2	89.4	89.4	91.9	91.1	90.2	87.8	99.2	97.6	99.2	100	100	99.2	98.4	96.7	**P1**	78.7
92.7	91.1	92.7	91.9	91.9	94.3	93.5	92.7	90.2	95.9	95.1	94.3	95.1	95.1	94.3	95.1	93.5	95.1	**M1**

Amino Acid Identity

TABLE 57

Nucleotide and deduced amino acid identity between isolates of HEV over 148 base (49 amino acid)* ORF 2 fragment

Nucleotide Identity

Ar1	91.8	87.8	81.6	82.7	83.7	80.6	82.7	87.8	80.6	80.6	80.6	80.6	80.6	80.0	82.7	79.6	80.6	80.6
100	**Ar2**	88.5	83.8	86.5	87.2	85.8	85.1	90.5	85.8	85.1	83.8	85.1	85.1	84.5	85.1	85.1	86.5	82.4
100	100	**Au1**	83.1	88.5	89.2	83.1	85.8	87.8	85.1	83.8	83.1	83.8	83.8	83.1	84.5	83.1	82.4	79.1
100	100	100	**G1**	87.2	87.8	84.5	85.1	85.1	84.5	82.4	82.4	83.1	83.1	82.4	83.1	82.4	83.8	81.1
100	100	100	100	**G2**	83.1	82.4	85.1	87.8	85.1	84.5	82.4	83.8	83.8	83.8	83.8	83.1	84.5	79.1
100	100	100	100	100	**It1**	87.8	85.8	85.8	83.8	83.1	82.4	83.1	83.1	82.4	83.8	81.8	82.4	79.7
96.9	98.0	98.0	98.0	98.0	98.0	**US-1**	93.9	90.5	79.0	78.4	76.4	77.0	77.0	76.4	77.0	78.4	77.7	79.7
96.9	98.0	98.0	98.0	98.0	98.0	100	**US-2**	91.2	82.4	80.4	79.7	80.4	80.4	79.3	80.4	81.8	81.1	81.8
96.9	98.0	98.0	98.0	98.0	98.0	100	100	**S1**	83.8	84.5	82.4	83.1	83.1	82.4	83.1	83.1	83.8	83.8
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	**B1**	98	94.6	95.3	95.3	94.6	96.6	97.3	93.9	82.4
96.9	95.9	95.9	95.9	95.9	95.9	93.9	93.9	93.9	98.0	**B2**	93.9	94.6	94.6	93.9	95.9	95.3	93.2	80.4
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	100	98.0	**C1**	98.0	98.0	96.6	96.6	91.9	96.6	81.8
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	100	98.0	100	**C2**	100	98.6	97.3	92.6	98.6	82.4
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	100	98.0	100	100	**C3**	98.6	97.3	92.6	98.6	82.4
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	100	98.0	100	100	100	**C4**	96.6	91.9	97.3	81.8
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	100	98.0	100	100	100	100	**I1**	93.9	95.9	83.8
93.8	95.9	95.9	95.9	95.9	95.9	93.9	93.9	93.9	98.0	95.9	98.0	98	98.0	98.0	98.0	**I2**	91.2	83.8
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	100	98.0	100	100	100	100	100	98.0	**P1**	83.1
96.9	98.0	98.0	98.0	98.0	98.0	95.9	95.9	95.9	95.9	93.9	95.9	95.9	95.9	95.9	95.9	93.9	95.9	**M1**

Amino Acid Identity

*Over 98 base (32 amino acid) fragment for Ar1

Comparisons of the ORF 1 and ORF 2 amplified sequences indicate that the isolates from the two patients from Argentina are quite distinct from each other, exhibiting 88.4 % and 91.8 % nucleotide sequence identity over these regions of ORF 1 and ORF 2, respectively. The value for ORF1 is lower than the lowest percent nucleotide identities between any Burmese-like isolates, which is 91.4%. for ORF 1. However for ORF2, the nucleotide identity of 91.8% between the two isolates from Argentina is in the range observed for identities between the Burmese-like isolates and ORF 2, which may be due to the shorter length of the fragment.

Phylogenetic analyses were performed as described in Example 7. The relative evolutionary distances between the viral sequences analyzed are readily apparent upon inspection of the unrooted phylogenetic trees generated from the pairwise distances, where the branch lengths are proportional to the relative genetic relationships between the isolates. The phylogenetic trees based on alignments of either 371 nucleotides from ORF 1 (Fig. 14), 148 nucleotides from ORF 2 (Fig. 15) which excludes Ar1, or 98 nucleotides from ORF 2 (Fig. 16), which includes Ar1, are quite similar in overall topology. The Burmese-like isolates and the Mexican isolate represent major branches at one end of the tree. The human US isolates form a distinct group distal to the Mexican and Burmese isolates. The swine HEV-like sequence is closely related to the US human isolates. The four European isolates and two Argentine isolates also form branches distal to the Mexican and Burmese isolates. The major branch between the US-type isolates, represented by the US, Greek, Italian, Austrian and Argentine isolates, and the Burmese-like and Mexican isolates is supported by a bootstrap value of 75.7% and greater in all trees.

Example 15: New Degenerate Primers

Degenerate primers derived from consensus oligonucleotide primers for HEV ORF 1 and ORF 2 were designed based on conserved regions between the full length sequences of isolates from Asia, Mexico, US as described in Example 11, as well as isolates from Greece and Italy. The ORF 1 primer is positioned within the methyltransferase region at nucleotides and 473-451 of the Burmese isolate (GenBank accession number M73218), and amplifies a product

417 nucleotides in length when used in combination with HEVConsORF 1-s1, SEQ ID NO:147; as described in Example11. The new ORF 1 primer combination includes:

HEVConsORF 1-s1; CTGGCATYACTACTGCYATTGAGC (SEQ ID NO:147); and
HEVConsORF 1N-a1; CCRTCRARRCARTAGGTGCGGTC (SEQ ID NO:255).

The new ORF 2 primer, at positions 6494-6470 of the Burmese isolate, produces a product 197 nucleotides in length when used in combination with HEVConsORF 2-s1; (SEQ ID NO:150); as described in Example11. The ORF 2 primers include:

HEVConsORF 2-s1; GACAGAATTRATTTCGTCGGCTGG (SEQ ID NO:150); and
HEVConsORF 2N-a1; CYTGYTCRTGYTGGTTRTCATAATC (SEQ ID NO:256).

For a second round of amplification, internal primers can be used to produce products 287 and 145 nucleotides in length for ORF 1 and ORF 2, respectively, as described in Example 11. The new combination of ORF 1 primers include:

HEVConsORF 1N-s2; CYGCCYTKGCGAATGCTGTGG (SEQ ID NO:257); and
HEVConsORF 1-a2; GGCAGWRTACCARCGCTGAACATC (SEQ ID NO:178).

The ORF 2 primers include:

HEVConsORF 2-s2; GTYGTCTCRGCCAATGGCGAGC (SEQ ID NO:152); and
HEVConsORF 2N-a2; GYTCRTGYTGRTTRTCATAATCCTG (SEQ ID NO:258).

PCR reactions contained 2 mM $MgCl_2$ and 0.5 μM of each oligonucleotide primer as per the manufacturer's instructions (Perkin-Elmer) and amplified using Touch-down PCR as described in Example 5. Amplified products were separated on a 1.5% agarose gel, stained with ethidium bromide, and analyzed for the presence of PCR products of the appropriate size. The primers were used to detect the presence of virus in serum containing HEV as described above and showed a marked increase in sensitivity over previous primers sets used in Example 11. These new primer combinations were found to be more sensitive with a number of different

variants of HEV that included two new isolates from Argentina, Ar1 and Ar2, and a new isolate from Austria, Au1 (see example 14 above), as well as isolates from Greece, G1, and Egypt, Eg46. The results are presented in Table 58 below in which NT represents samples not tested, "-" represents no product band detectable by ethidium bromide staining, "+/-"represents a weak product band detectable by ethidium bromide staining, and "2+", "3+" and "4+" represent increasing amounts of product as detected by ethidium bromide staining.

TABLE 58

	ORF1				ORF2			
	PCR1		PCR2		PCR1		PCR2	
SAMPLE	**Old Set**	**New Set**	**Old Set**	**New Set**	**Old Set**	**New Set**	**Old Set**	**New Set**
Ar 1	-	2+	2+	4+	2+	4+	3+	4+
Ar 2	-	2+	3+	4+	+/-	+/-	-	3+
Au 1	-	2+	3+	4+	-	3+	3+	4+
Eg46	NT	NT	NT	NT	-	3+	3+	4+
G1	-	-	2+	-	3+	3+	3+	4+

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A method of detecting the presence of a US-type or US-subtype hepatitis E virus (HEV) or a naturally occurring variant thereof in a test sample, the method comprising the steps of:

(a) contacting the sample with a binding partner that binds specifically to a marker for said virus, which if present in the sample binds to the binding partner to produce a marker-binding partner complex, and

(b) detecting the presence of said complex, the presence of said complex being indicative of the presence of said virus in the sample.

2. The method of claim 1, wherein said marker is an antibody capable of binding said virus.

3. The method of claim 2, wherein said antibody is an immunoglobulin G or an immunoglobulin M.

4. The method of claim 2, wherein said binding partner is an isolated polypeptide chain.

5. The method of claim 4, wherein said polypeptide chain is immobilized on a solid support.

6. The method of claim 4, wherein said binding partner is a polypeptide chain selected from the group consisting of SEQ ID NOS:91, 92, and 93, including naturally occurring variants thereof.

7. The method of claim 4, wherein said binding partner is a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:173 or SEQ ID NO:175.

8. The method of claim 4, where said binding partner is a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:174 or SEQ ID NO:176.

9. The method of claim 4, wherein said binding partner is a polypeptide chain selected from the group consisting of SEQ ID NOS:166, 167 and 168, including naturally occurring variants thereof.

10. The method of claim 4, wherein said binding partner is a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:223.

11. The method of claim 4, wherein said binding partner is a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:224.

12. The method of claim 1, wherein said binding partner is an isolated antibody capable of binding specifically to a polypeptide chain selected from the group consisting of SEQ ID NOS:91, 92, 93, 166, 167, and 168, including naturally occurring variants thereof.

13. The method of claim 12, wherein said antibody is a monoclonal antibody.

14. The method of claim 1, wherein said marker is a polypeptide chain.

15. The method of claim 14, wherein said polypeptide chain is selected from the group consisting of SEQ ID NOS:91, 92, and 93, including naturally occurring variants thereof.

16. The method of claim 14, wherein said polypeptide chain comprises the amino acid sequence set forth in SEQ ID NO:173 or SEQ ID NO:175.

17. The method of claim 14, wherein said polypeptide chain comprises the amino acid sequence set forth in SEQ ID NO:174 or SEQ ID NO:176.

18. The method of claim 14, wherein said polypeptide chain is selected from the group consisting of SEQ ID NOS:166, 167, and 168, including naturally occurring variants thereof.

19. The method of claim 14, wherein said polypeptide chain comprises the amino acid sequence set forth in SEQ ID NO:223.

20. The method of claim 14, wherein said polypeptide chain comprises the amino acid sequence set forth in SEQ ID NO:224.

21. The method of claim 1, wherein said marker is a nucleic acid sequence defining at least a portion of a genome of said virus, or a complementary strand thereof.

22. The method of claim 1 wherein said binding partner is an isolated nucleic acid sequence that is capable of hybridizing under specific hybridization conditions to the nucleic acid sequences set forth in SEQ ID NOS:89 and 164.

23. The method of claim 1 wherein said binding partner is selected from the group consisting of SEQ ID NOS:126, 128, 147, 148, 150, 152, 177, 178, 255, 256, 257, and 258.

24. The method of claim 1 wherein said binding partner is an isolated polypeptide chain.

25. The method of claim 1 wherein said test sample is a mammalian cell line.

26. The method of claim 41 wherein said mammalian cell line is a human fetal kidney cell line.

27. A method of detecting the presence of a hepatitis E virus (HEV) in a test sample, the method comprising the steps of:

(a) contacting the sample with a binding partner selected from the group consisting of SEQ ID NOS: 126, 128, 147, 148, 150, 152, 177, 178, 255, 256, 257, and 258 that binds specifically to a marker for said virus, which if present in the sample binds to the binding partner to produce a marker-binding partner complex, and

(b) detecting the presence of said complex, the presence of said complex being indicative of the presence of said virus in the sample.

28. An isolated polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:223 and SEQ ID NO:224.

29. An isolated antibody capable of binding specifically to a polypeptide chain selected from the group consisting of a polypeptide encoded by an ORF 1 sequence of a US-type or a US-subtype HEV, a polypeptide encoded by an ORF 2 sequence of a US-type or a US-subtype HEV, and a polypeptide encoded by an ORF 3 sequence of a US-type or a US-subtype HEV.

30. An isolated antibody capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:173, SEQ ID NO:175 or SEQ ID NO:224.

31. An isolated antibody capable of binding specifically to a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:174, SEQ ID NO:176 or SEQ ID NO:223.

32. The isolated antibody of claim 30, wherein said antibody, under similar conditions, has a lower affinity for a polypeptide chain comprising the amino acid sequence set forth in SEQ ID NO:169 or 171.

33. The isolated antibody of claim 31, wherein said antibody, under similar conditions, has a lower affinity for a polypeptide chain comprising the amino acid sequence set forth SEQ ID NO:170 or 172.

34. The isolated antibody of claim 29 further comprising a detectable moiety.

35. An isolated nucleic acid sequence defining at least a portion of an ORF 1, ORF 2 or ORF 3 sequence of a US-type or US-subtype hepatitis E virus, or a sequence complementary thereto.

36. An isolated nucleic acid sequence capable of hybridizing under specific hybridization conditions to the nucleotide sequence set forth in SEQ ID NOS:89 and 164.

37. A vector comprising the isolated nucleic acid sequence of claim 35.

38. A host cell containing the vector of claim 37.

39. A method of immunizing a mammal against a US-type or US-subtype HEV, the method comprising administering to the mammal the polypeptide of claim 28 in an amount sufficient to stimulate the production of an antibody capable of binding specifically to the US-type or US-subtype hepatitis E virus.

40. A method of immunizing a mammal against a US-type or US-subtype HEV 1, the method comprising administering to said mammal the antibody of claim 29 in an amount sufficient to immunize said mammal against the US-type or US-subtype hepatitis E virus.

41. A method of immunizing a mammal against a US-type or US-subtype HEV 1, the method comprising administering to said mammal the antibody of claim 30 in an amount sufficient to immunize said mammal against the US-type or US-subtype hepatitis E virus.

42. A method of immunizing a mammal against a US-type or US-subtype HEV 1, the method comprising administering to said mammal the antibody of claim 31 in an amount sufficient to immunize said mammal against the US-type or US-subtype hepatitis E virus.

43. A method of immunizing a mammal against a US-type or US-subtype HEV, the method comprising administering to said mammal the host cell of claim 38 in an amount sufficient to immunize said mammal against the US-type or US-subtype hepatitis E virus.

ABSTRACT OF THE DISCLOSURE

Disclosed herein are methods and compositions for detecting the presence in a sample of a US-type or a US-subtype hepatitis E virus, including naturally occurring variants thereof. In particular, the invention provides nucleic acid sequences corresponding to the genome of the US-type or US-subtype hepatitis E virus, amino acid sequences, including epitope sequences, encoded by the genomes of such viruses, and antibodies that bind specifically to such amino acid sequences. The invention further provides methods and compositions for immunizing individuals against infection by, or for treating individuals already infected with such a virus.

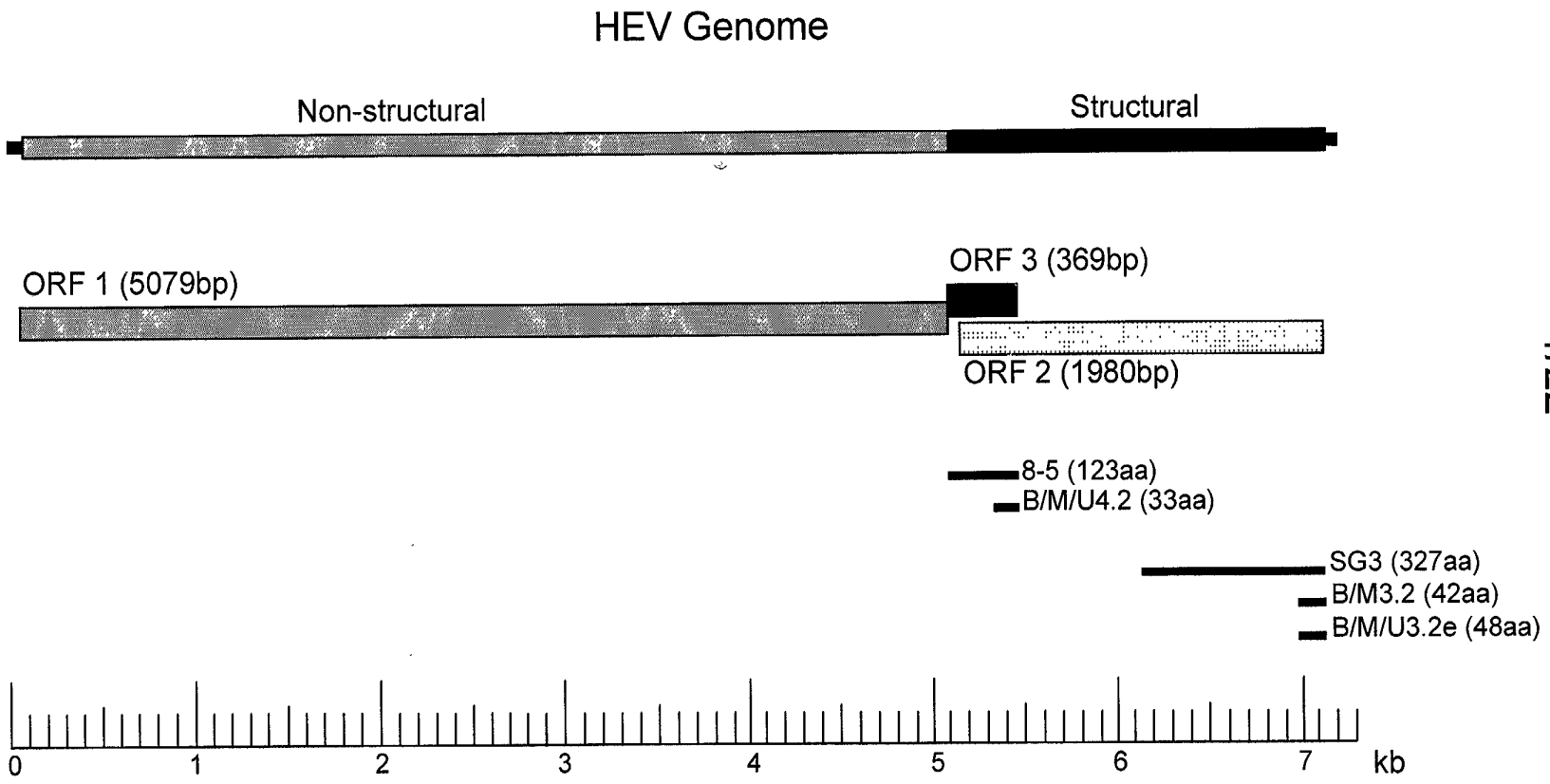
HEV Genome
Non-structural
Structural
ORF 1 (5079bp)
ORF 3 (369bp)
ORF 2 (1980bp)
8-5 (123aa)
B/M/U4.2 (33aa)
SG3 (327aa)
B/M3.2 (42aa)
B/M/U3.2e (48aa)
0
1
2
3
4
5
6
7
kb

Figure 1

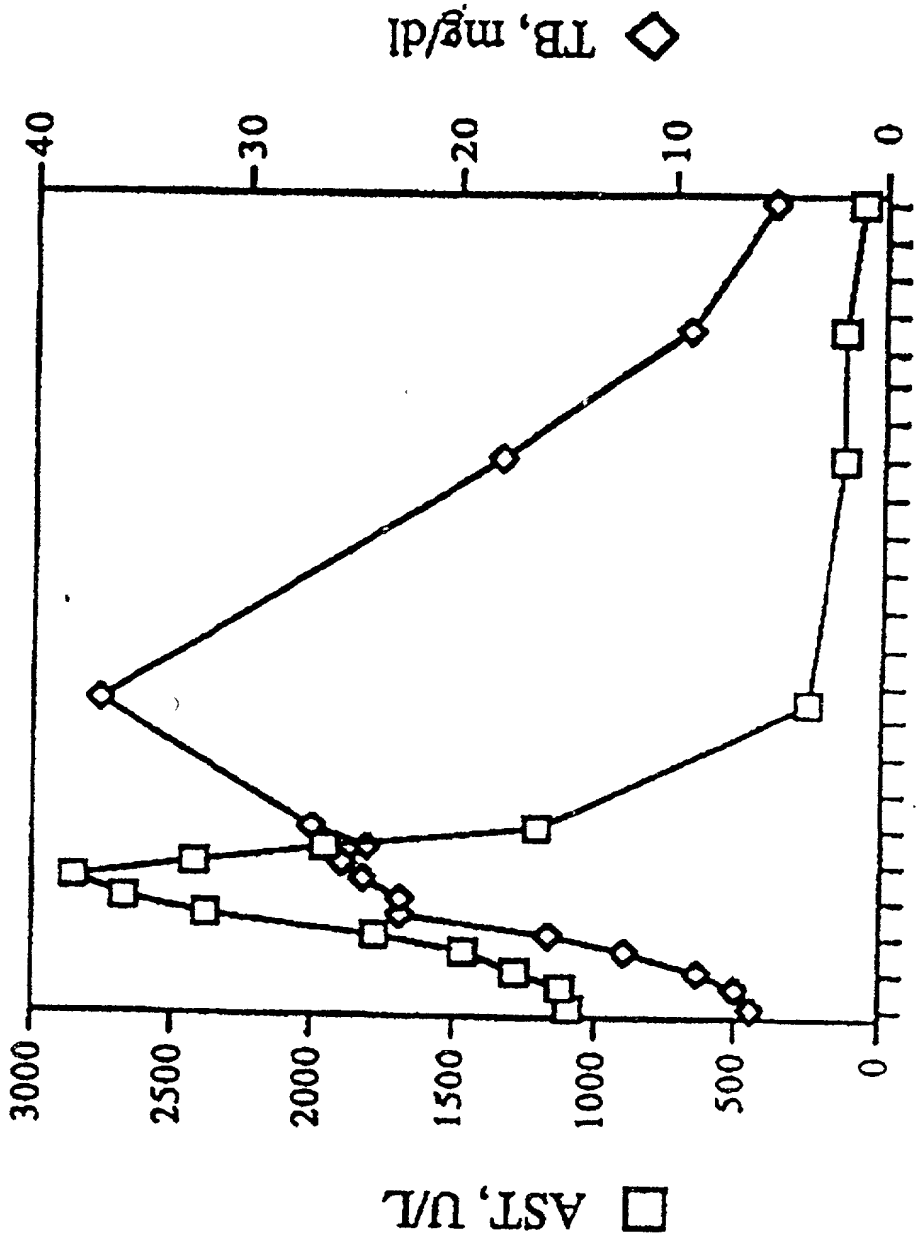

Figure 2

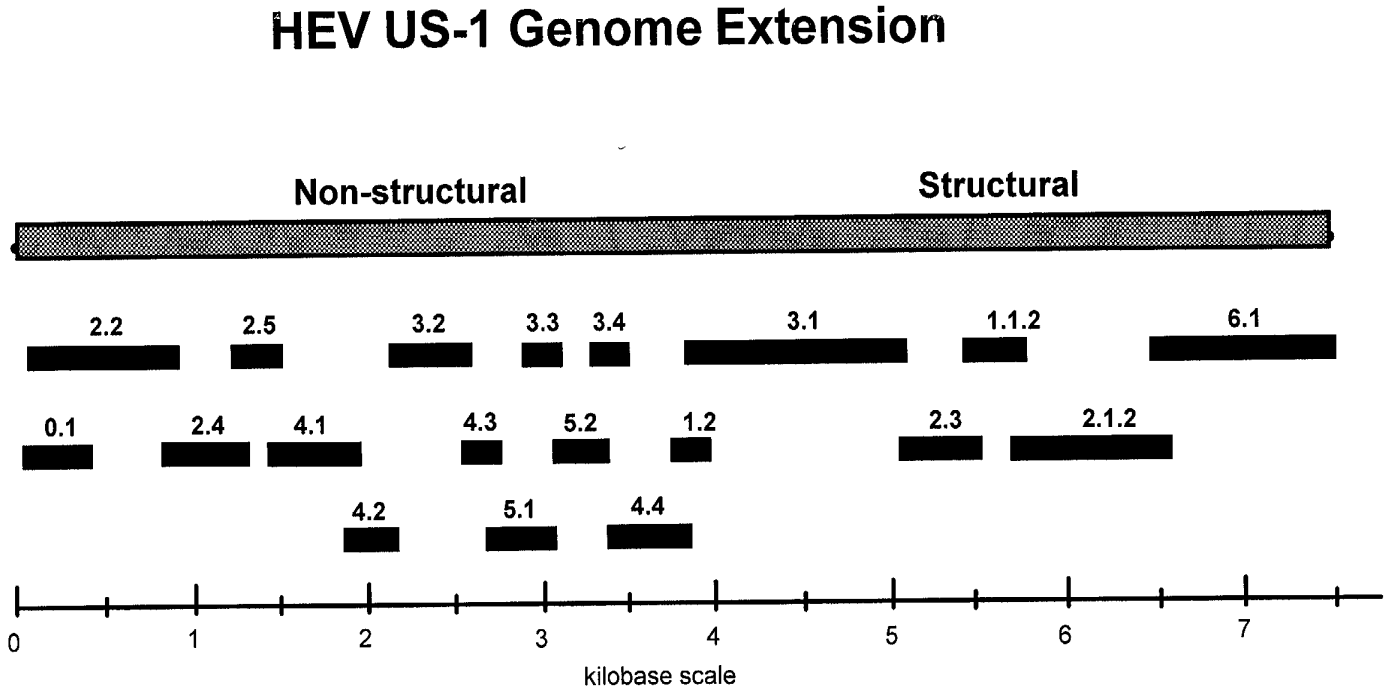
HEV US-1 Genome Extension
Non-structural
Structural
2.2
2.5
3.2
3.3
3.4
3.1
1.1.2
6.1
0.1
2.4
4.1
4.3
5.2
1.2
2.3
2.1.2
4.2
5.1
4.4
0
1
2
3
4
5
6
7
kilobase scale

Figure 3

Extension of HEV US-2

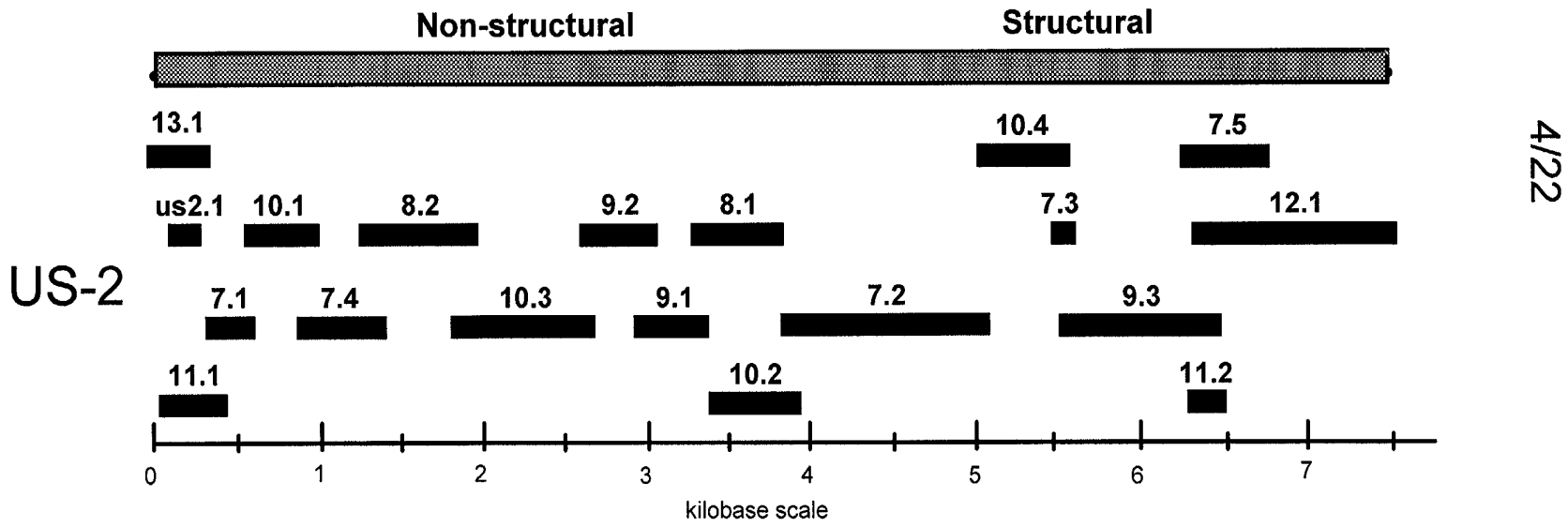

Figure 4

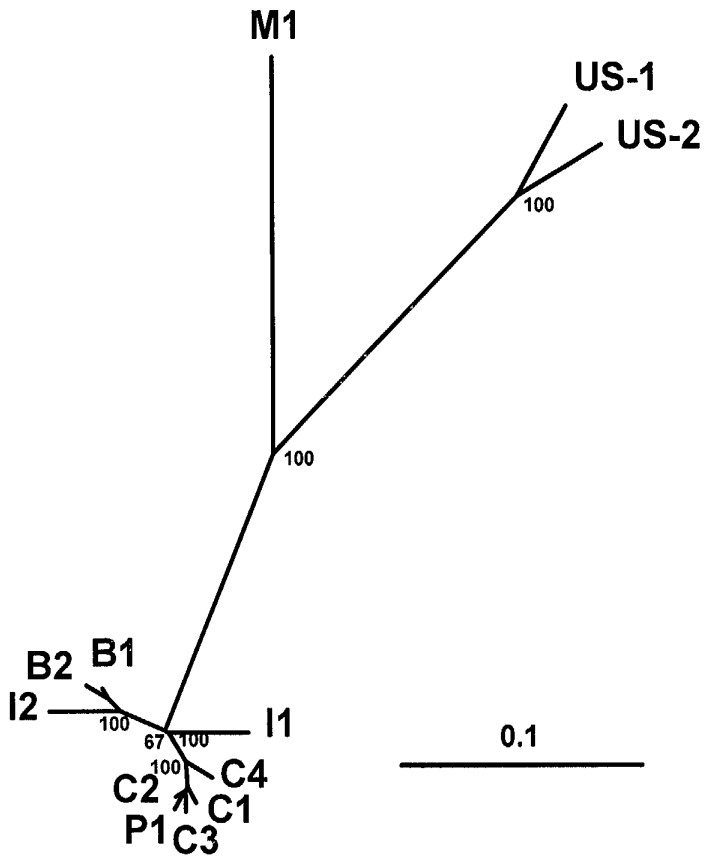

Figure 5

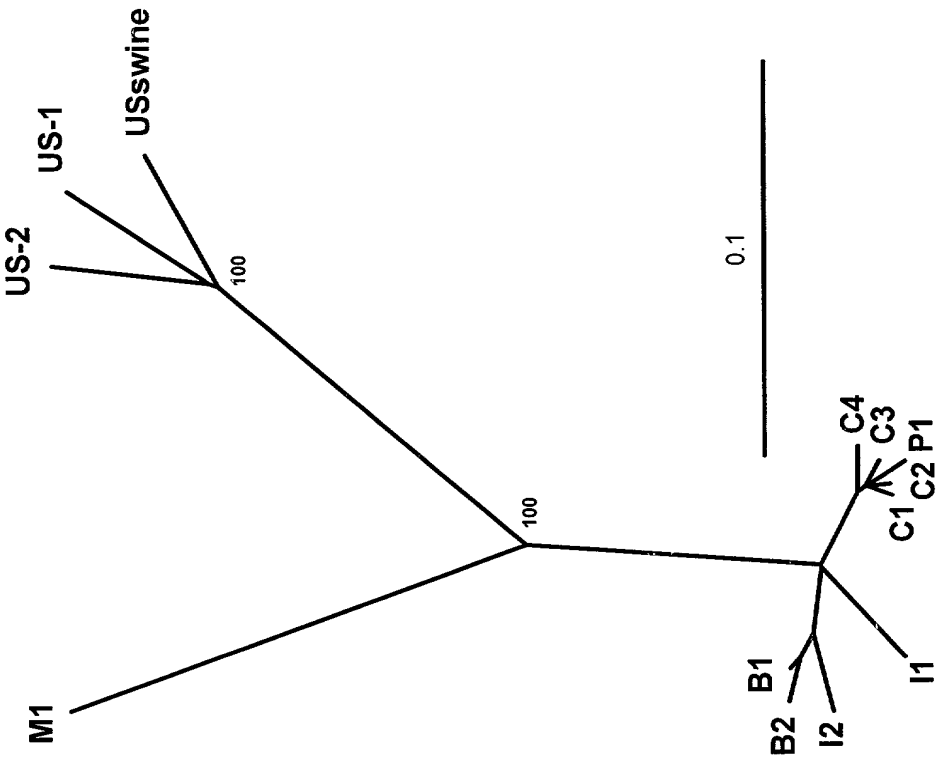

Figure 6

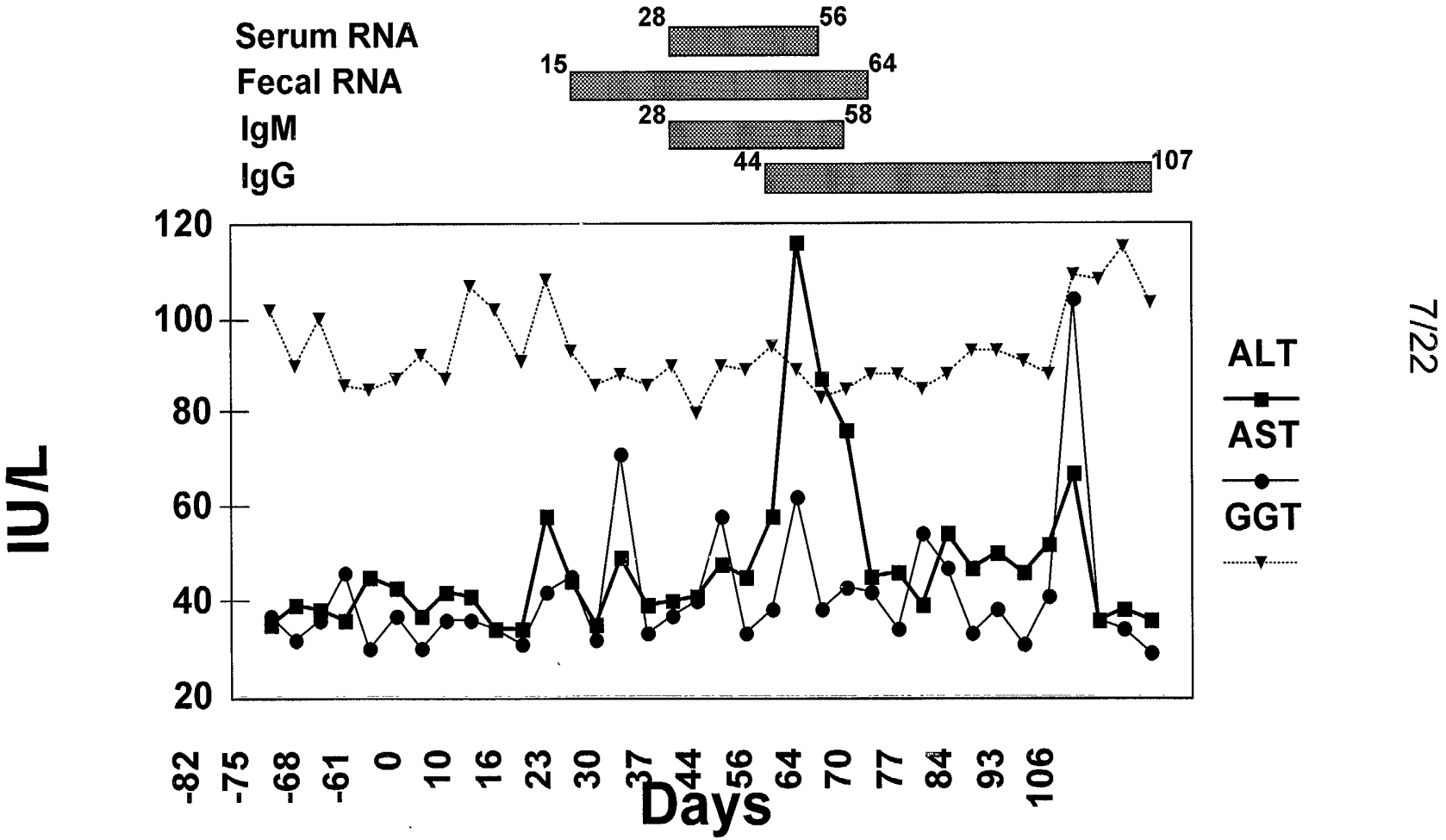
Serum RNA
Fecal RNA
IgM
IgG
28
56
15
64
28
58
44
107
120
100
80
60
40
20
IU/L
ALT
AST
GGT
-82
-75
-68
-61
0
10
16
23
30
37
44
56
64
70
77
84
93
106
Days

Figure 7

Extension of Z12

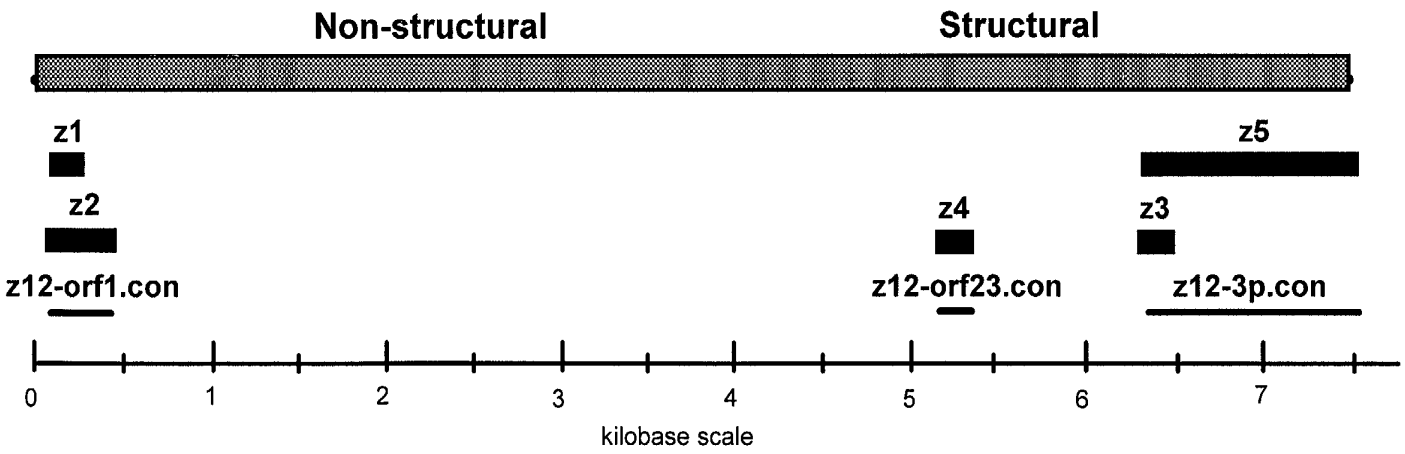

Figure 8

FIGURE 9A

```
                  51                                                    100
  5p.pile{hpesvp} GGCTCCTGGC ATCACTACTG CTATTGAGCA GGCTGCTCTA GCAGCGGCCA
 5p.pile{hpeuigh} GGCTCCTGGC ATCACTACTG CTATTGAGCA GGCTGCTCTA GCAGCGGCCA
    5p.pile{hpea} GGCTCCTGGC ATCACTACTG CTATTGAGCA GGCTGCTCTA GCAGCGGCCA
 5p.pile{840455p} ~~~~CCTGGC ATTACTACTG CCATTGAGCA GGCTGCTCTG GCTGCGGCCA
 5p.pile{hpenssp} GGCTCCTGGC ATCACTACTG CTATTGAGCA AGCAGCTCTA GCAGCGGCCA
        Consensus ----CCTGGC AT-ACTACTG C-ATTGAGCA -GC-GCTCT- GC-GCGGCCA

                  101                                                   150
  5p.pile{hpesvp} ACTCTGCCCT GGCGAATGCT GTGGTAGTTA GGCCTTTTCT CTCTCACCAG
 5p.pile{hpeuigh} ATTCTGCCCT TGCGAATGCT GTGGTAGTTA GGCCTTTTCT CTCTCACCAG
    5p.pile{hpea} ACTCTGCCCT TGCGAATGCT GTGGTAGTTA GGCCTTTTCT CTCTCACCAG
 5p.pile{840455p} ATTCTGCCTT GGCGAATGCT GTGGTGGTTC GGCCGTTTTT ATCTCGCGTG
 5p.pile{hpenssp} ACTCCGCCCT TGCGAATGCT GTGGTGGTCC GGCCTTTCCT TTCCCATCAG
        Consensus A-TC-GCC-T -GCGAATGCT GTGGT-GT-- GGCC-TT--T -TC-C----G

                  151                                                   200
  5p.pile{hpesvp} CAGATTGAGA TCCTCATTAA CCTAATGCAA CCTCGCCAGC TTGTTTTCCG
 5p.pile{hpeuigh} CAGATTGAGA TCCTTATTAA CCTAATGCAA CCTCGCCAGC TTGTTTTCCG
    5p.pile{hpea} CAGATTGAGA TCCTTATTAA CCTAATGCAA CCTCGCCAGC TTGTTTTCCG
 5p.pile{840455p} CAAACCGAGA TTCTTATTAA TTTGATGCAA CCCCGGCAGT TGGTTTTCCG
 5p.pile{hpenssp} CAGGTTGAGA TCCTTATAAA TCTCATGCAA CCTCGGCAGC TGGTGTTTCG
        Consensus CA----GAGA T-CT-AT-AA --T-ATGCAA CC-CG-CAG- T-GT-TT-CG
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FIGURE 9B

```
                201                                                 250
 5p.pile{hpesvp}  CCCCGAGGTT TTCTGGAATC ATCCCATCCA GCGTGTCATC CATAACGAGC
5p.pile{hpeuigh}  CCCCGAGGTT TTCTGGAACC ACCCCATCCA GCGTGTCATC CATAATGAGC
   5p.pile{hpea}  CCCCGAGGTT TTCTGGAACC ATCCCATCCA GCGTGTTATC CATAATGAGC
5p.pile{840455p}  CCCTGAGGTA CTTTGGAATC ACCCTATCCA GCGGGTTATA CATAATGAAT
5p.pile{hpenssp}  TCCTGAGGTT TTTTGGAATC ACCCGATTCA ACGTGTTATA CATAATGAGC
       Consensus  -CC-GAGGT- -T-TGGAA-C A-CC-AT-CA -CG-GT-AT- CATAA-GA--

                251                                                 300
 5p.pile{hpesvp}  TGGAGCTTTA CTGCCGCGCC CGCTCCGGCC GCTGTCTTGA AATTGGCGCC
5p.pile{hpeuigh}  TGGAGCTTTA CTGTCGCGCC CGCTCCGGCC GCTGCCTTGA AATTGGTGCC
   5p.pile{hpea}  TGGAGCTTTA CTGTCGCGCC CGCTCCGGCC GCTGCCTCGA AATTGGTGCC
5p.pile{840455p}  TAGAACAGTA CTGCCGGGCT CGGGCTGGTC GTTGCTTGGA GGTTGGAGCT
5p.pile{hpenssp}  TTGAGCAGTA TTGCCGTGCT CGCTCGGGTC GCTGCCTTGA GATTGGAGCC
       Consensus  T-GA-C--TA -TG-CG-GC- CG--C-GG-C G-TG--T-GA --TTGG-GC-

                301                                                 350
 5p.pile{hpesvp}  CATCCCCGCT CAATAAATGA TAATCCTAAT GTGGTCCACC GCTGCTTCCT
5p.pile{hpeuigh}  CACCCTCGCT CAATAAACGA CAATCCTAAT GTGGTCCACC GCTGCTTCCT
   5p.pile{hpea}  CACCCCCGCT CAATAAATGA CAATCCTAAT GTGGTCCACC GTTGCTTCCT
5p.pile{840455p}  CACCCAAGAT CCATTAATGA CAACCCCAAC GTTCTGCATC GGTGTTTCCT
5p.pile{hpenssp}  CACCCACGCT CCATTAATGA TAATCCTAAT GTCCTCCATC GCTGCTTTCT
       Consensus  CA-CC--G-T C-AT-AA-GA -AA-CC-AA- GT--T-CA-C G-TG-TT-CT
```

FIGURE 9C

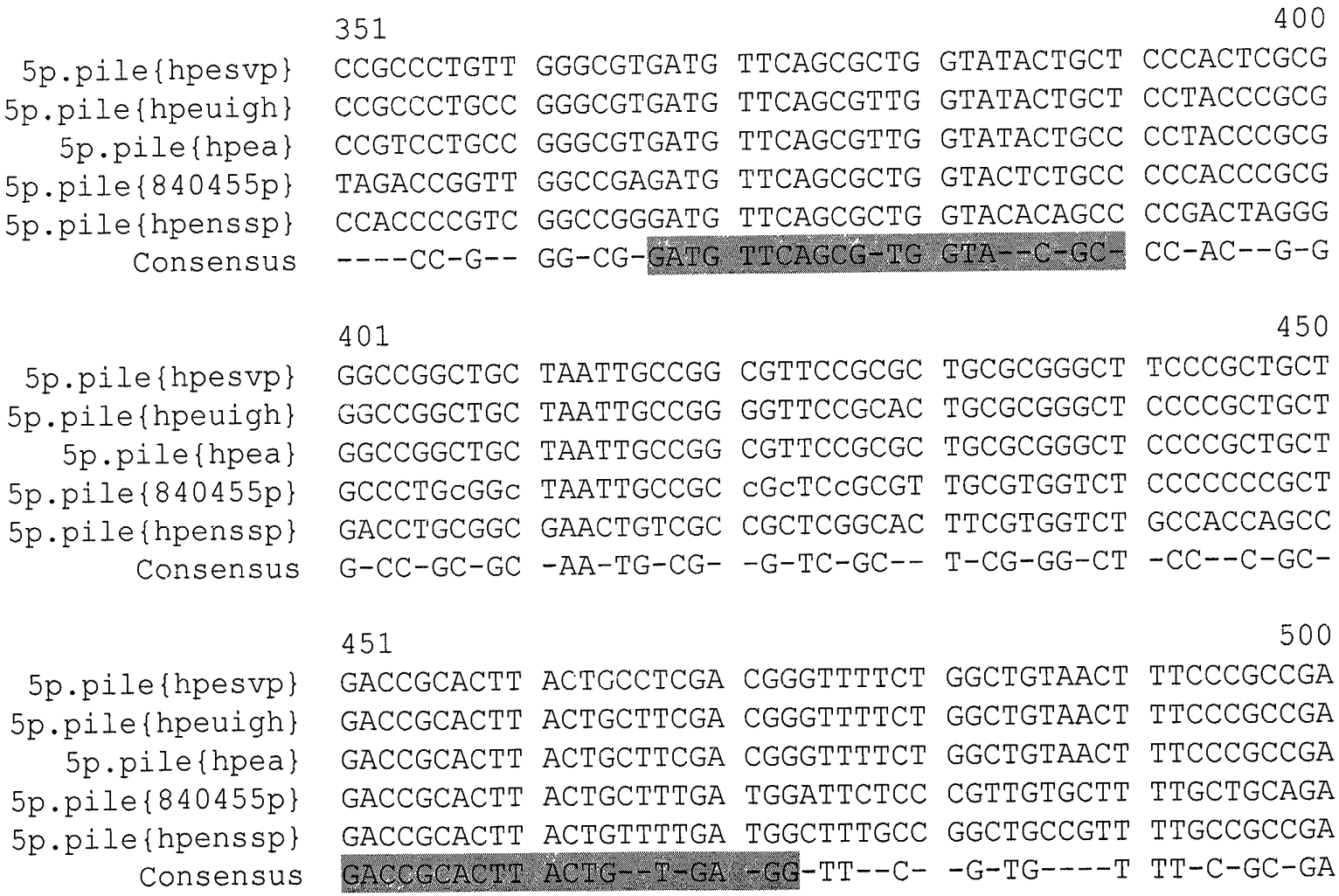
351 400
5p.pile{hpesvp} CCGCCCTGTT GGGCGTGATG TTCAGCGCTG GTATACTGCT CCCACTCGCG
5p.pile{hpeuigh} CCGCCCTGCC GGGCGTGATG TTCAGCGTTG GTATACTGCT CCTACCCGCG
5p.pile{hpea} CCGTCCTGCC GGGCGTGATG TTCAGCGTTG GTATACTGCC CCTACCCGCG
5p.pile{840455p} TAGACCGGTT GGCCGAGATG TTCAGCGCTG GTACTCTGCC CCCACCCGCG
5p.pile{hpenssp} CCACCCCGTC GGCCGGGATG TTCAGCGCTG GTACACAGCC CCGACTAGGG
Consensus ----CC-G-- GG-CG-GATG TTCAGCG-TG GTA--C-GC- CC-AC--G-G
401 450
5p.pile{hpesvp} GGCCGGCTGC TAATTGCCGG CGTTCCGCGC TGCGCGGGCT TCCCGCTGCT
5p.pile{hpeuigh} GGCCGGCTGC TAATTGCCGG GGTTCCGCAC TGCGCGGGCT CCCCGCTGCT
5p.pile{hpea} GGCCGGCTGC TAATTGCCGG CGTTCCGCGC TGCGCGGGCT CCCCGCTGCT
5p.pile{840455p} GCCCTGcGGc TAATTGCCGC cGcTCcGCGT TGCGTGGTCT CCCCCCCGCT
5p.pile{hpenssp} GACCTGCGGC GAACTGTCGC CGCTCGGCAC TTCGTGGTCT GCCACCAGCC
Consensus G-CC-GC-GC -AA-TG-CG- -G-TC-GC-- T-CG-GG-CT -CC--C-GC-
451 500
5p.pile{hpesvp} GACCGCACTT ACTGCCTCGA CGGGTTTTCT GGCTGTAACT TTCCCGCCGA
5p.pile{hpeuigh} GACCGCACTT ACTGCTTCGA CGGGTTTTCT GGCTGTAACT TTCCCGCCGA
5p.pile{hpea} GACCGCACTT ACTGCTTCGA CGGGTTTTCT GGCTGTAACT TTCCCGCCGA
5p.pile{840455p} GACCGCACTT ACTGCTTTGA TGGATTCTCC CGTTGTGCTT TTGCTGCAGA
5p.pile{hpenssp} GACCGCACTT ACTGTTTTGA TGGCTTTGCC GGCTGCCGTT TTGCCGCCGA
Consensus GACCGCACTT ACTG--T-GA -GG-TT--C- -G-TG----T TT-C-GC-GA

FIGURE 9D

```
                1451                                                1500
   3p.pile{hpea}  ACTGAGTCAG TGAAGCCAGT GCTTGACCTG ACAAATTCAA TTCTGTGTCG
3p.pile{hpeuigh}  ACTGAGTCGG TGAAGCCAGT GCTCGACTTG ACAAATTCAA TCCTGTGTCG
 3p.pile{hpesvp}  ACTGAGTCAG TAAAACCAGT GCTCGACTTG ACAAATTCAA TCTTGTGTCG
3p.pile{hpenssp}  ACAGAGTCTG TTAAGCCTAT ACTTGACCTT ACACACTCAA TTATGCACCG
3p.pile{840453p}  ACAGAGACTA TTAAACCTGT ACTTGATCTC ACAAATTCCA TCATACAGCG
       Consensus  AC-GAG-C-- T-AA-CC--T -CT-GA--T- ACA-A-TC-A T--T----CG

                1501                                                1550
   3p.pile{hpea}  GGTGGAATGA ATAACATGTC TTTTGCTGCG CCCATGGGTT CGCGACCATG
3p.pile{hpeuigh}  GGTGGAATGA ATAACATGTC TTTTGCTGCG CCCATGGGTT GGCGACCATG
 3p.pile{hpesvp}  GGTGGAATGA ATAACATGTC TTTTGCTGCG CCCATGGGTT CGCGACCATG
3p.pile{hpenssp}  GTCTGAATGA ATAACATGTG GTTTGCTGCG CCCATGGGTT CGCCACCATG
3p.pile{840453p}  GGTGGAATGA ATAACATGTC TTTTGCATCG CCCATGGGAT C...ACCATG
       Consensus  G---GAATGA ATAACATGT- -TTTGC--CG CCCATGGG-T ----ACCATG

                1551                                                1600
   3p.pile{hpea}  CGCCCTCGGC CTATTTTGCT GTTGCTCCTC ATGTTTCTGC CTATGCTGCC
3p.pile{hpeuigh}  CGCCCTCGGC CTATTTTGCT GTTGCTCCTC ATGTTTCTGC CTATCGTGCC
 3p.pile{hpesvp}  CGCCCTCGGC CTATTTTGTT GCTGCTCCTC ATGTTTTTGC CTATGCTGCC
3p.pile{hpenssp}  CGCCCTAGGC CTCTTTTGCT GTTGTTCCTC TTGTTTCTGC CTATGTTGCC
3p.pile{840453p}  CGCCCTAGGG CTGTTCTGTT GTTGTTCCTC ATGTTTCTGC CTATGCTGCC
       Consensus  CGCCCT-GG- CT-TT-TG-T G-TG-TCCTC -TGTTT-TGC CTAT--TGCC
```

FIGURE 9E

```
                1601                                                1650
   3p.pile{hpea}  CGCGCCACCG CCCGGTCAGC CGTCTGGCCG CCGTCGTGGG CGGCGCAGCG
3p.pile{hpeuigh}  CGCGCCACCG CCCGGTCAGC CGTCTGGCCG CCGTCGTGGG CGGCGCAGCG
 3p.pile{hpesvp}  CGCGCCACCG CCCGGTCAGC CGTCTGGCCG CCGTCGTGGG CGGCGCAGCG
3p.pile{hpenssp}  CGCGCCACCG ACCGGTCAGC CGTCTGGCCG CCGTCGTGGG CGGCGCAGCG
3p.pile{840453p}  CGCGCCACCG GCCGGTCAGC CGTCTGGCCG TCGCCGTGGG CGGCGCAGCG
       Consensus  CGCGCCACCG -CCGGTCAGC CGTCTGGCCG -CG-CGTGGG CGGCGCAGCG

                1651                                                1700
   3p.pile{hpea}  GCGGTTCCGG CGGTGGTTTC TGGGGTGACC GGGTTGATTC TCAGCCCTTC
3p.pile{hpeuigh}  GCGGTTCCGG CGGTGGTTTC TGGGGTGACC GGGTTGATTC TCAGCCCTTC
 3p.pile{hpesvp}  GCGGTTCCGG CGGTGGTTTC TGGGGTGACC GGGTTGATTC TCAGCCCTTC
3p.pile{hpenssp}  GCGGTACCGG CGGTGGTTTC TGGGGTGACC GGGTTGATTC TCAGCCCTTC
3p.pile{840453p}  GCGGTGCCGG CGGTGGTTTC TGGAGTGACA GGGTTGATTC TCAGCCCTTC
       Consensus  GCGGT-CCGG CGGTGGTTTC TGG-GTGAC- GGGTTGATTC TCAGCCCTTC

                1701                                                1750
   3p.pile{hpea}  GCAATCCCCT ATATTCATCC AACCAACCCC TTCGCCCCCG ATGTCACCGC
3p.pile{hpeuigh}  GCAATCCCCT ATATTCATCC AACCAACCCC TTCGCCCCCG ATGTCACCGC
 3p.pile{hpesvp}  GCAATCCCCT ATATTCATCC AACCAACCCC TTCGCCCCCG ATGTCACCGC
3p.pile{hpenssp}  GCAATCCCCT ATATTCATCC AACCAACCCC TTTGCCCCAG ACGTTGCCGC
3p.pile{840453p}  GCCCTCCCCT ATATTCATCC AACCAACCCC TTCGCCGCCG ATGTCGTTTC
       Consensus  GC--TCCCCT ATATTCATCC AACCAACCCC TT-GCC-C-G A-GT-----C
```

FIGURE 9F

```
                   2651                                                 2700
  3p.pile{hpea}    AGCGCTTACC CTGTTTAACC TTGCTGACAC CCTGCTTGGC GGTCTACCGA
3p.pile{hpeuigh}   AGCGCTTACC CTGTTTAACC TTGCTGACAC CCTGCTTGGC GGTCTACCGA
 3p.pile{hpesvp}   AGCCCTCACC CTGTTCAACC TTGCTGACAC TCTGCTTGGC GGCCTGCCGA
3p.pile{hpenssp}   AGCTCTAACA TTACTTAACC TTGCTGACAC GCTCCTCGGC GGGCTCCCGA
3p.pile{840453p}   TGCCCtGACT CTGTTTAATC TTGCTGATaC GCTTCTTGGT GGTTTACCGA
       Consensus   -GC-CT-AC- -T--T-AA-C TTGCTGA-AC -CT-CT-GG- GG--T-CCGA

                   2701                                                 2750
  3p.pile{hpea}    CAGAATTGAT TTCGTCGGCT GGTGGCCAGC TGTTCTACTC TCGCCCCGTC
3p.pile{hpeuigh}   CAGAATTGAT TTCGTCGGCT GGTGGCCAGC TGTTCTACTC TCGCCCCGTC
 3p.pile{hpesvp}   CAGAATTGAT TTCGTCGGCT GGTGGCCAGC TGTTCTACTC CCGTCCCGTT
3p.pile{hpenssp}   CAGAATTAAT TTCGTCGGCT GGCGGGCAAC TGTTTTATTC CCGCCCGGTT
3p.pile{840453p}   CAGAATTGAT TTCGTCGGcT GGGGGTCAAC TGTTTTACTC CCGCCCTGTT
       Consensus   CAGAATT-AT TTCGTCGGCT GG-GG-CA-C TGTT-TA-TC -CG-CC-GT-

                   2751                                                 2800
  3p.pile{hpea}    GTCTCAGCCA ATGGCGAGCC GACTGTTAAG CTGTATACAT CTGTGGAGAA
3p.pile{hpeuigh}   GTCTCAGCCA ATGGCGAGCC GACTGTTAAG CTGTATACAT CTGTAGAGAA
 3p.pile{hpesvp}   GTCTCAGCCA ATGGCGAGCC GACTGTTAAG TTGTATACAT CTGTAGAGAA
3p.pile{hpenssp}   GTCTCAGCCA ATGGCGAGCC AACCGTGAAG CTCTATACAT CAGTGGAGAA
3p.pile{840453p}   GTcTCgGCCA ATGGCGAgCC AAcAGTAaAG TTATACACAT CTGTtGAgAA
       Consensus   GTCTC-GCCA ATGGCGAGCC -AC-GT-AAG -T-TA-ACAT C-GT-GAGAA
```

FIGURE 9G

```
                   2801                                                   2850
   3p.pile{hpea}   TGCTCAGCAG GATAAGGGTA TTGCAATCCC GCATGACATC GACCTCGGGG
 3p.pile{hpeuigh}  TGCTCAGCAG GATAAGGGTA TTGCAATCCC GCATGACATC GACCTCGGGG
  3p.pile{hpesvp}  TGCTCAGCAG GATAAGGGTA TTGCAATCCC GCATGACATT GACCTCGGAG
 3p.pile{hpenssp}  TGCTCAGCAG GATAAGGGTG TTGCTATCCC CCACGATATC GATCTTGGTG
 3p.pile{840453p}  TGCgCAgCAA gACAAGGGca TcacCaTTCC ACACGACATA gATTTAGGTG
        Consensus  TGC-CAGCA- GA-AAGGG-- T--C-AT-CC -CA-GA-AT- GA--T-GG-G

                   2851                                                   2900
   3p.pile{hpea}   AATCCCGTGT AGTTATTCAG GATTATGACA ACCAACATGA GCAGGACCGA
 3p.pile{hpeuigh}  AATCTCGAGT TGTTATTCAG GATTATGACA ACCAACATGA GCAGGACCGG
  3p.pile{hpesvp}  AATCTCGTGT GGTTATTCAG GATTATGATA ACCAACATGA ACAAGATCGG
 3p.pile{hpenssp}  ATTCGCGTGT GGTCATTCAG GATTATGACA ACCAGCATGA GCAGGATCGG
 3p.pile{840453p}  ACTCCCGTGT GGTTAtCCAG gattATgATa ACcagcacga acaAgaTcgA
        Consensus  A-TC-CG-GT -GT-AT-CAG GATTATGA-A ACCA-CA-GA -CA-GA-CG-
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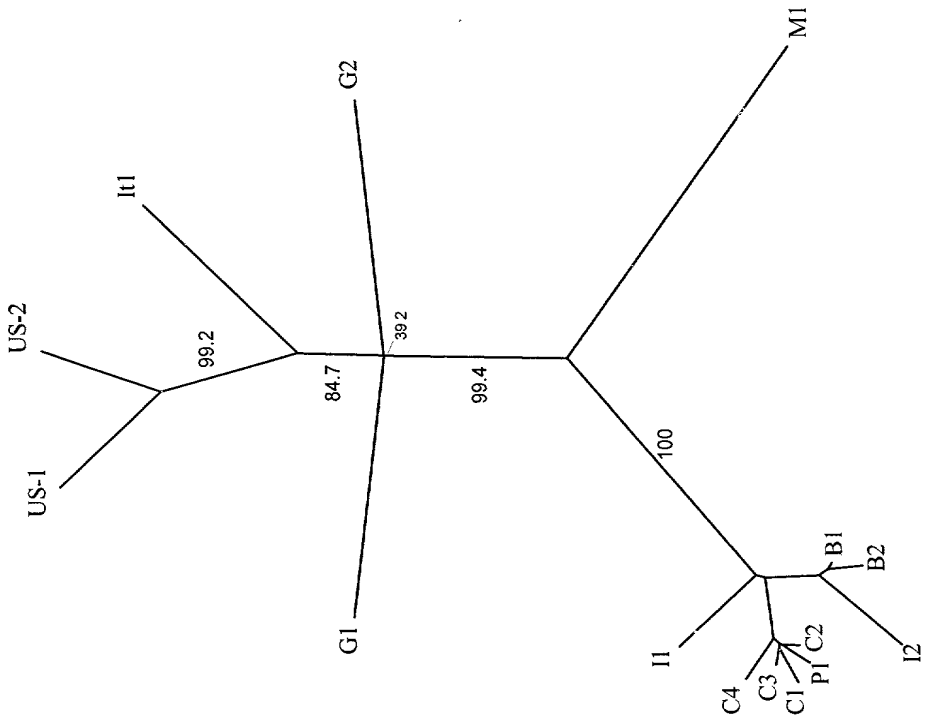
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G2
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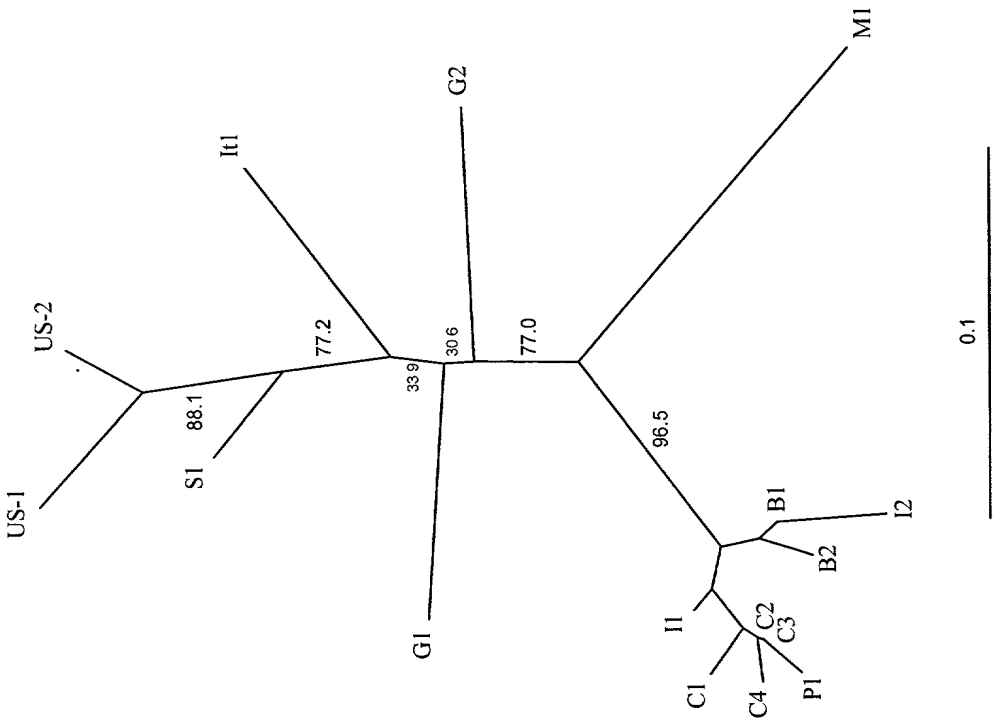
P1
C2
B1
B2
I2
0.1

Figure 10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S1
88.1
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It1
33.9
30.6
G2
G1
77.0
M1
96.5
B1
I2
B2
I1
C2
C3
C1
C4
P1
0.1

Figure 11

Figure 12A

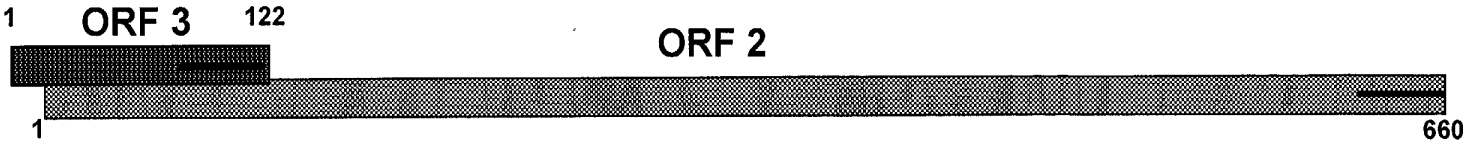
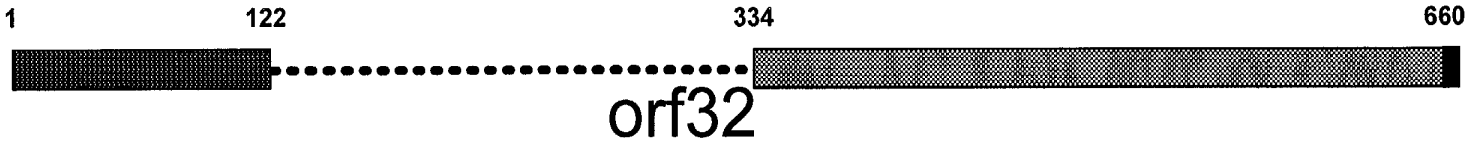
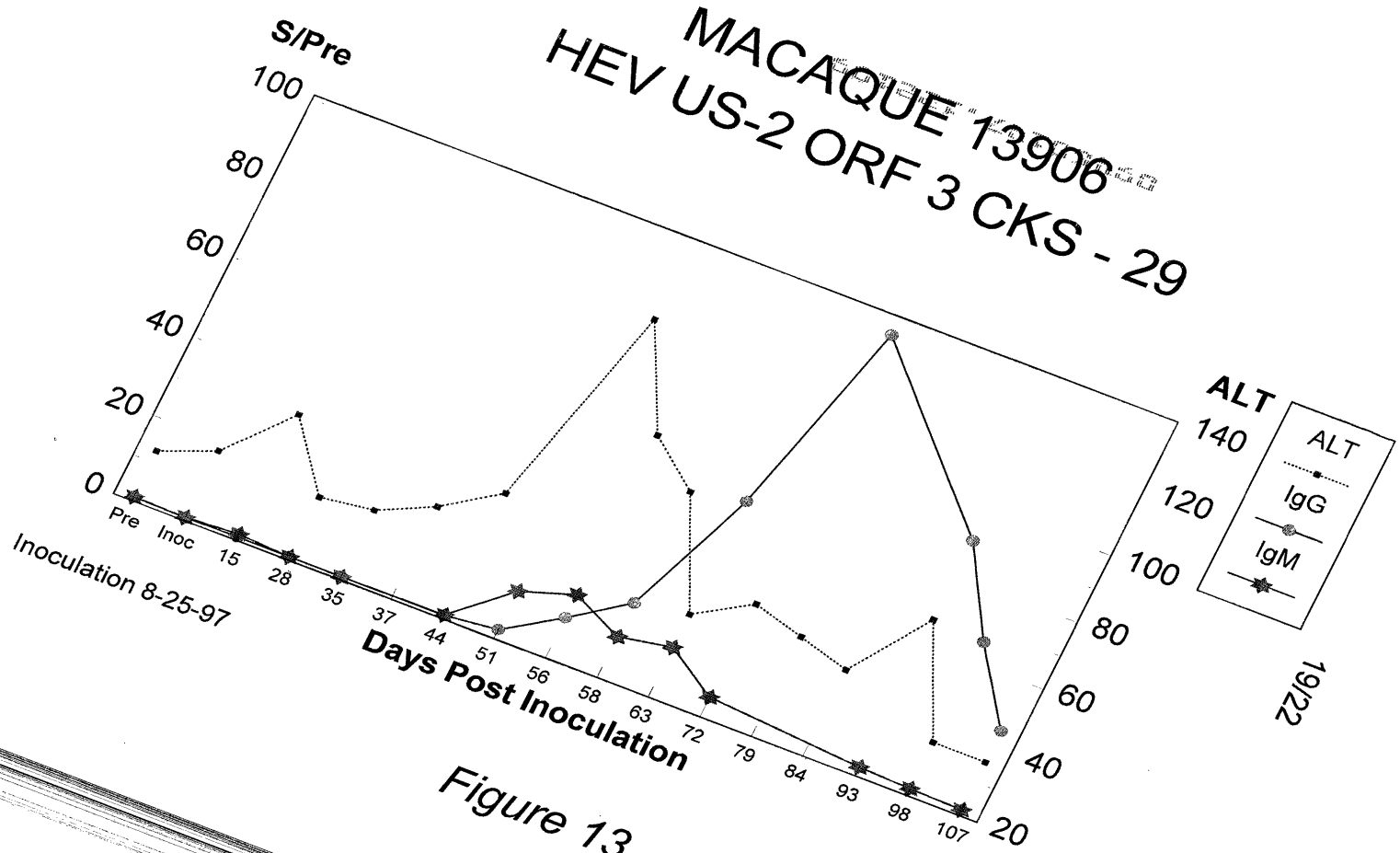


Figure 12B

Figure 12C

Figure 12D

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S/Pre
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80
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40
20
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140
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40
20
ALT
IgG
IgM
Pre
Inoc
15
28
35
37
44
51
56
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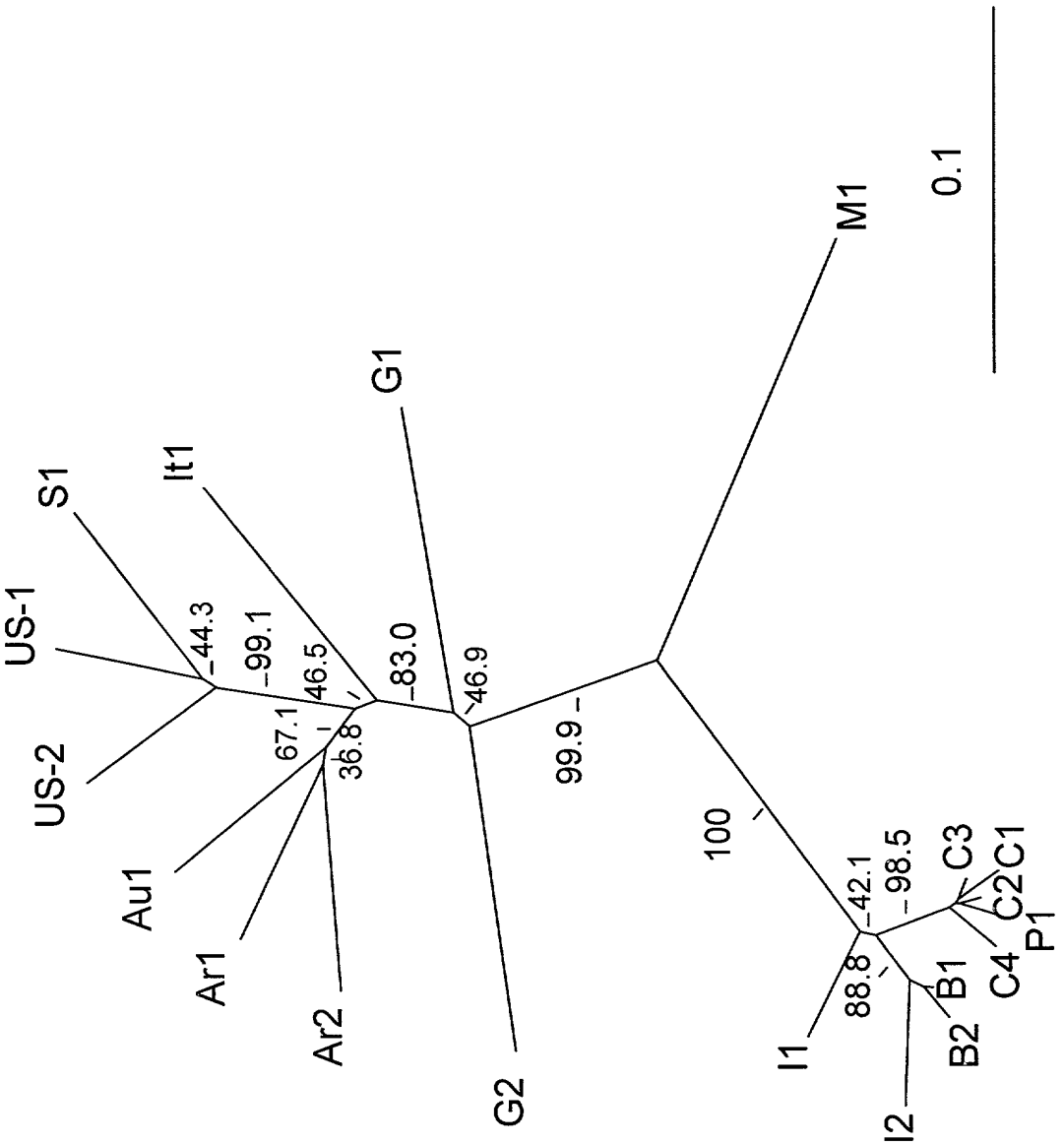
107
Inoculation 8-25-97
Days Post Inoculation
19/22

Figure 13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S1
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G1
M1
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US-2
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I1
I2
B2
B1
C4
C2
C1
C3
P1
44.3
99.1
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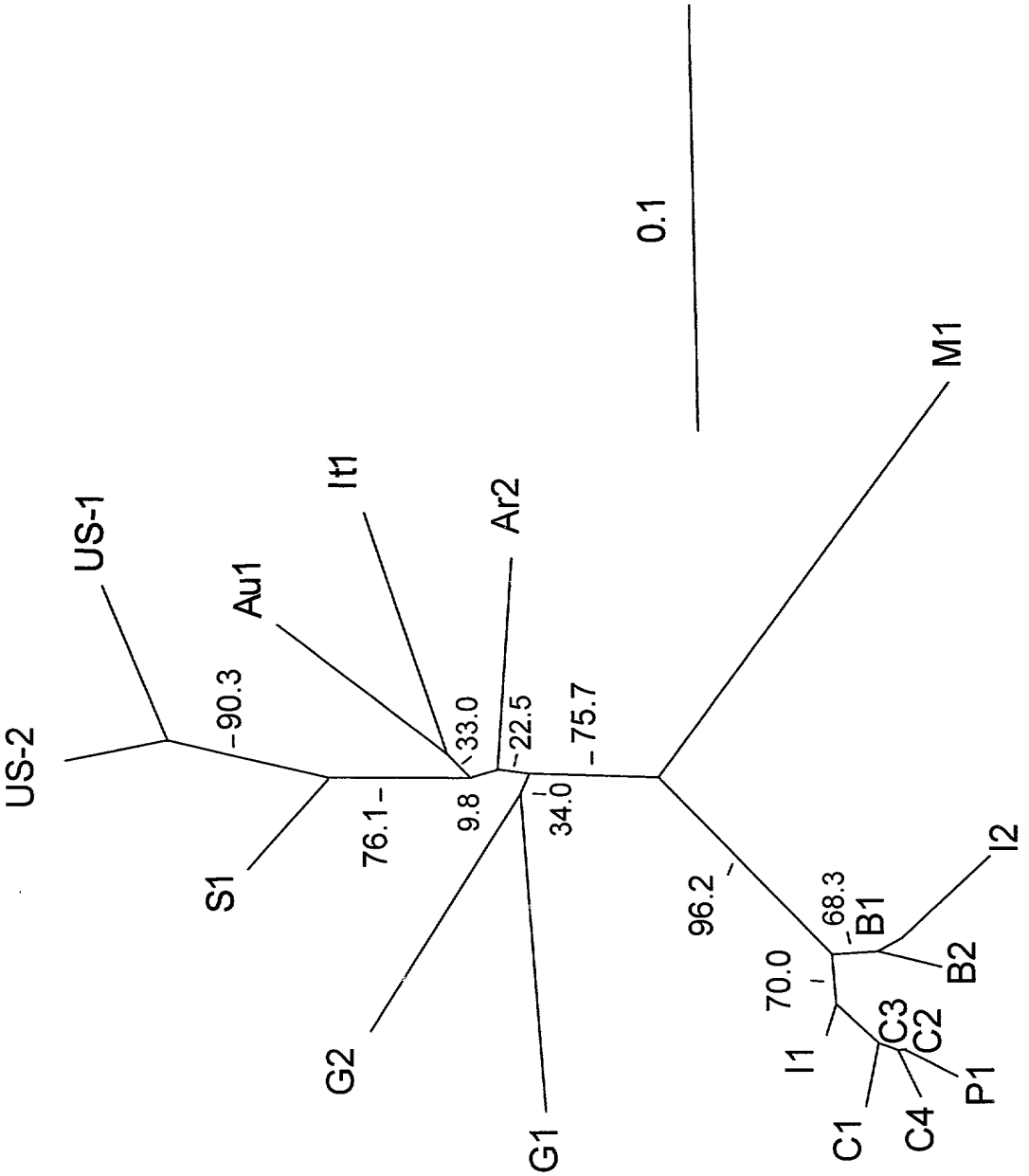
67.1
36.8
99.9
100
42.1
98.5
88.8

Figure 14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It1
Ar2
M1
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90.3
33.0
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75.7
76.1
9.8
34.0
96.2
68.3
70.0
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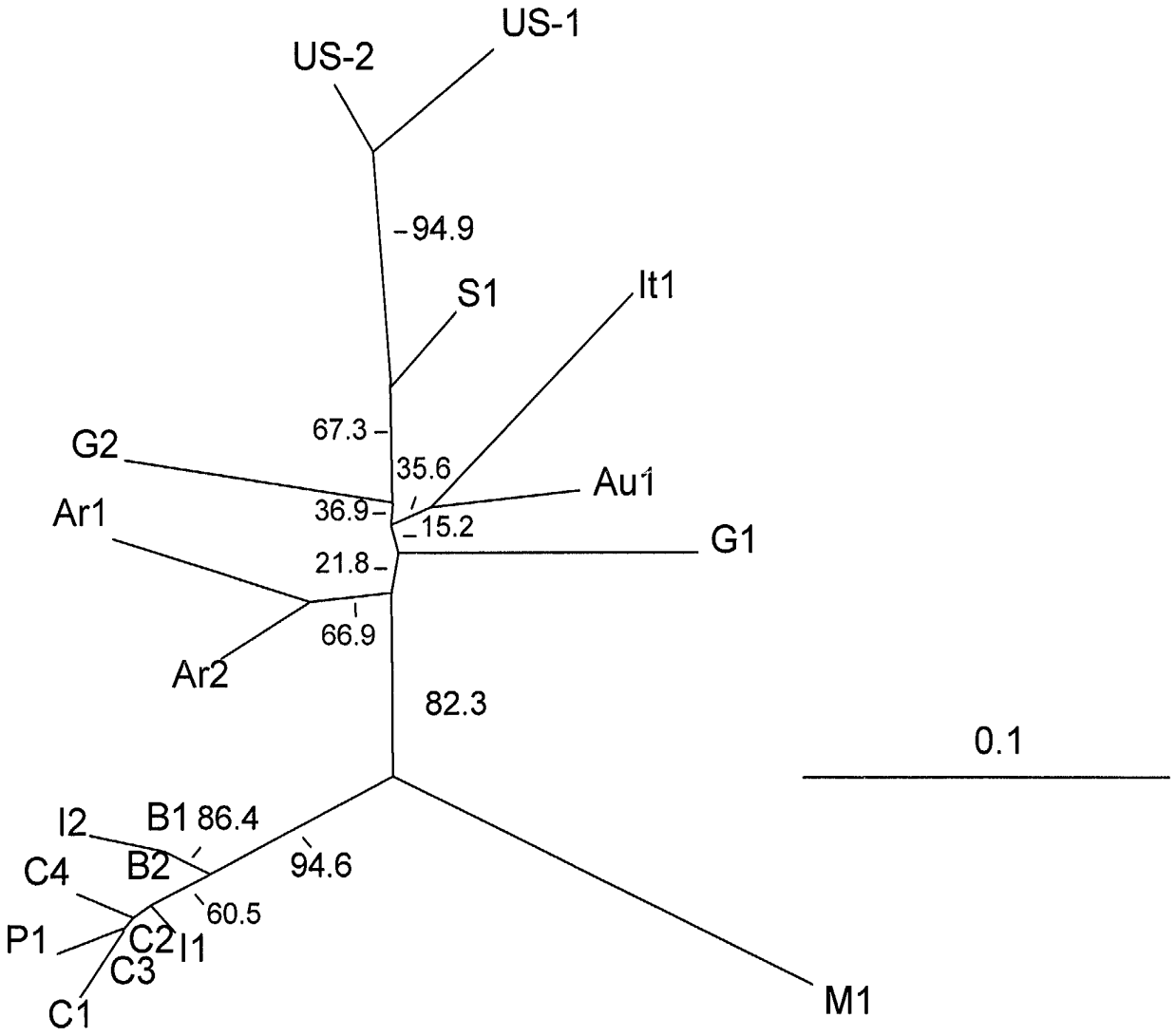
G2
G1
I1
B1
B2
I2
C1
C4
C3
C2
P1

Figure 15


US-1
US-2
94.9
S1
It1
67.3
G2
35.6
Au1
36.9
Ar1
15.2
G1
21.8
66.9
Ar2
82.3
0.1
I2
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86.4
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B2
94.6
60.5
P1
C2
I1
C3
C1
M1

Figure 16

SEQUENCE LISTING

<110> Schlauder, George G
Erker, James C
Desai, Suresh M
Dawson, George J
Mushawar, Isa K

<120> METHODS AND COMPOSITIONS FOR DETECTING HEPATITIS E VIRUS

<130> 6232.US.01

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<150> US 09/173,141
<151> 1998-10-15

<160> 258

<170> PatentIn Ver. 2.0

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accgagattc ttattaattt gatgcaaccc cggcagttgg ttttccgccc tgaggtactt 180

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gctggtcgtt gcttggaggt tggagctcac ccaagatcca ttaatgacaa ccccaacgtt 300

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<211> 251
<212> DNA
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<220>
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ccgttgcccc ccgtcgtcga tctaccccag ctggggctgc gccgctaact gccatatcac 180

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agtataacct a 251

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tactgatgtc aggatcttgg tccagcccgg tatagcctcc gagttagtca tccctagtga 420

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ggaggctacc tccggtctgg taatgctttg tattcatggc tcccctgtta actcctacac 540

taatacacct tacaccggtg cattggggct tcttgatttt gcattagaac ttgaatttag 600

aaatttgaca cccgggaaca ctaacacccg tgtttcccgg tatactagca cagcccgcca 660

ccggctgcgc cgcggtgctg atgggaccgc tgagctcacc accacagcag ccacacgctt 720

catgaaggat ttgcatttta ctggtacgaa cggcgttggt gaggtgggtc gtggtattgc 780

cctgactctg tttaatcttg ctgatacgct tcttggtggt ttaccgacag aattgatttc 840

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<210> 26
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<212> DNA
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ggttggccga gatgttcagc gctggtactc tgcccccacc cgcggccctg cggctaattg 360

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atcgat 846

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<223> Description of Artificial Sequence: Primer 3900a

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<210> 31
<211> 25
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<220>
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uf-orf2/3 a3

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acatgtcttt tgcatcgccc atgggatcac catgcgccct agggctgttc tgttgttgtt 180

cctcatgttt ctgcctatgc tgcccgcgcc accggccggt cagccgtctg gccgtcgccg 240

tgggcggcgc agcggcggtg ccggcggtgg tttctggagt gacagggttg attctcagcc 300

cttcgccctc ccctatattc atccaaccaa ccccttcgcc gccgatgtcg tttcacaacc 360

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gtcc 424

<210> 34
<211> 24
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<223> Description of Artificial Sequence: Primer 426-a3

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<223> Description of Artificial Sequence: Primer 167-s2

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aatgttgcgc aggtctgtgt tgatgttgtg tcccgtgtct atggagtcag ccccgggctc 1140

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<211> 25
<212> DNA
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<220>
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<210> 40

<211> 22
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<223> Description of Artificial Sequence: Primer 1300a

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<220>
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gcgttttgct gttcacggct catgacttac ctccgtggta ttagttacaa ggtcactgtc 240

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actgcagctt atttgactat ttgccatcag cgttatctcc gcacccaggc gatatccaag 360

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<213> Artificial Sequence

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<210> 44
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<213> Hepatitis E virus

<220>
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ctagacccca gggtacttgt ttttgatgag tcagtaccat gccgctgtag gacgtttttg 180

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<223> us1 gap-s1

<400> 45
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<210> 46
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<223> us1 gap-a0.5

<400> 46
gctgcaagac cctcacgcat gatg 24

<210> 47
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<223> us1 gap-s2

<400> 47
cggattatgg ttacaccctg agg 23

<210> 48
<211> 25
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<223> us1 gap-a1

<400> 48
attcagttgg gtaaaacgct tctgg 25

<210> 49
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<212> DNA
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<400> 49
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aacatctggc ttttctagtg atttctcccc ccctgaagcg gccgctcctg ctatggctgc 180

taccccgggg ctgccccatt ctaccccacc tgttagcgat atttgggtgc taccaccgcc 240

ctcagaggag tttcaggttg atgcagcacc tgtgcccccc gcccctgacc ctgctggatt 300

gcccggtccc gttgtgctta ccccccccc ccctccccct gtgcataagc catcaatacc 360

cccgccttcc cgtaaccgtc gtctcctcta tacctatcct gacggcgcta aggtgtatgc 420

agggtcactg tttgaatcag actgtgactg gctggttaat gcctcaaacc cgggccatcg 480

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tgaat 545

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<223> us1-2600s

<400> 50
gtgctcacca taactgagga cacg 24

<210> 51
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<223> us1-2600a

<400> 51
cgctgcatat gtaacagcaa cagg 24

<210> 52
<211> 344
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<223> us1-344

<400> 52
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gctacagagg tcggccgtgc ttgtgccggt tgcaccatca gccctggcat tgtgcactat 120

cagtttaccg ccggggtccc gggctcgggc aagtcaaggt ccatacaaca gggagatgtc 180

gatgtggtgg ttgtgcccac ccgggagctt cgtaatagtt ggcgccgccg gggttttgcg 240

gccttcacac cccacacagc ggcccgtgtt actatcggcc gccgcgttgt gattgatgag 300

gctccatctc tcccgccaca cctgttgctg ttacatatgc agcg 344

<210> 53
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<213> Hepatitis E virus

<220>
<223> us1 3200s

<400> 53
gccgatgtgt gcgagctcat acg 23

<210> 54
<211> 25
<212> DNA
<213> Hepatitis E virus

<220>
<223> us1 3200a

<400> 54
atgattgtgg tctctgtgaa ggtgg 25

<210> 55

<211> 194
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<223> us1-194

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ctacggtccc tgttttggaa tgaaccggcc attggccaga agttggttyt cacgcaggcg 120

gcaaaggctg ctaaccctgg tgcgattacg gtccacgaag ctcagggtgc caccttcaca 180

gagaccacaa tcat 194

<210> 56
<211> 23
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<220>
<223> HEV216-s1

<400> 56
cagtaccatg ccgctgtagg acg 23

<210> 57
<211> 26
<212> DNA
<213> Hepatitis E virus

<220>
<223> us2-733a1

<400> 57
ccattagatg aaatctttac ctgcag 26

<210> 58
<211> 26
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<220>
<223> HEV216-s2

<400> 58
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<210> 59
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<210> 60
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<212> DNA
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acgaggccta tgagggttct gaggtcgacc cggctgaacc tgcacatctt gatgtttctg 180

ggacttacgc cgtccacggg caccagcttg aggccctcta tagggcactt aatgtcccac 240

aagatattgc cgctcgagct tcccgactaa cggcaactgt tgagctcgtt gcaagtccag 300

accgcttaga gtgccgcacc gtgctcggta ataagacctt ccggacgacg gtggtcgacg 360

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agtctatggg ggccgggtcg cacagcctca cttatgagct cacc 464

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<223> us1 733s1

<400> 61
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<210> 62
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<223> us2851-r2

<400> 62
ccagaggttg accaggttcg gg 22

<210> 63
<211> 24
<212> DNA
<213> Hepatitis E virus

<220>
<223> us1 733s2

<400> 63
ccgtgctcgg taataagacc ttcc 24

<210> 64
<211> 433
<212> DNA
<213> Hepatitis E virus

<220>
<223> us1-432

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ctggcggatt atggttacac cctgaggggt tgctgggtat tttcccccct ttctcccctg 360

ggcatatctg ggagtctgcg aacccctttt gcggggaggg gactttgtat acccgaacct 420

ggtcaacctc tgg 433

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<223> us2851-f1

<400> 65
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<210> 66
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<212> DNA
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<223> us1 430-a1

<400> 66
cgtgtcctca gttatggtga gcac 24

<210> 67
<211> 26
<212> DNA
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<223> us1 430-a2

<400> 67
tattagcctc aaaccaattt gcagcg 26

<210> 68
<211> 382
<212> DNA
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<223> us1-382

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ggtcttgcag catacacctt gaccccgcgc cctatcattc atgcagtcgc tcccgattat 180

agggttgagc agaacccgaa gaggcttgag gcagcgtacc gtgaaacttg ttcccgtcgt 240

ggcaccgctg cctacccgct tttgggttcg ggtatatacc aggtccctgt tagcctcagt 300

tttgatgcct gggaacgtaa tcaccgcccc ggcgatgagc tttacttgac cgagcccgct 360

gcaaattggt ttgaggctaa ta 382

<210> 69
<211> 22
<212> DNA
<213> Hepatitis E virus

<220>
<223> us2-579-s1

<400> 69
cagaccacga gccgtgtgct ac 22

<210> 70
<211> 25
<212> DNA

<213> Hepatitis E virus

<220>
<223> JE hev167-a1

<400> 70
ccaacacact atcggacaca gtgag 25

<210> 71
<211> 22
<212> DNA
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<223> us2-579-s2

<400> 71
gctgctaagg ctgccaaccc tg 22

<210> 72
<211> 24
<212> DNA
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<223> JE hev167-a2

<400> 72
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<210> 73
<211> 451
<212> DNA
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<223> us1-579wb

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catgctatag ttgcacttac tcgccacact gagaagtgtg ttatcctgga tgcccccggc 180

ctgcttcgtg aggtcggcat ttcggatgtg attgtcaaca acttttttcct tgctggtggc 240

gaggtcggcc rccaccgccc ttctgtgata cctcgcggta accctgatca aaacctcggg 300

actttacagg ccttcccgcc gtcctgtcaa attagtgctt accatcagtt ggctgaggaa 360

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ggcctgctct acatgccaca ggagctcact g 451

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<220>
<223> us2-430s1

<400> 74
ggtatatacc aggtccctgt tagc 24

<210> 75
<211> 22
<212> DNA
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<223> us2-482-a1

<400> 75
ccgctgtgtg aggtgtgaag gc 22

<210> 76
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<212> DNA
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<223> us2-430s2

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gttagcctca gttttgatgc ctgg 24

<210> 77
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<223> us2-482-a2

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<210> 78
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accgagcccg ctgcaaattg gtttgaggct aataagccgg cgcagccggt gctcaccata 120

actgaggaca cggcccgtac ggccaacctg gcattggaga ttgatgccgc tacagaggtc 180

ggccgtgctt gtgccggttg caccatcagc cctggcattg tgcactatca gtttaccgcc 240

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gtgcccaccc gggagctccg taacagctgg cgtc 334

<210> 79
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<400> 79
gatgtcgatg tggtggttgt gcc 23

<210> 80
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<400> 80
gtaatcgcac cagggttggc agc 23

<210> 81
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<400> 81
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<210> 82
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<223> JE us2-579-a2

<400> 82
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<210> 83
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<223> us1-482wb

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gttgctgtta catatgcagc gggcctcctc ggtccatctc ctcggtgacc caaatcagat 180

ccctgctatt gattttgagc acgccggcct ggtccctgcg atccgtcccg agcttgcgcc 240

aacgagctgg tggcrcgtta cacaccgttg cccggccgat gtgtgcgagc tcatacgcgg 300

agcctaccct aaaatccaga ccacgagccg tgtgctacgg tccctgtttt ggaatgaacc 360

ggccattggc cagaagttgg ttytcacgca ggctgctaag gctgccaacc ctg 413

<210> 84
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: oligo dT
adapter primer

<400> 84
ggccacgcgt cgactagtac ttttttttttt ttttttt 37

<210> 85
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: AUAP primer

<400> 85
ggccacgcgt cgactagtac 20

<210> 86
<211> 22

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer
df-orf3-s1

<400> 86
gcgttggtga ggtgggtcgt gg 22

<210> 87
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer
df-orf3-s2

<400> 87
cgcttcttgg tggtttaccg acag 24

<210> 88
<211> 960
<212> DNA
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<220>
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<400> 88
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aaattgaagg ttgattaccg ccctatcggg ctgtatgctg gggtggtggt ggcccccggt 4800

ttggggacac tgcccgatgt ggtgcgtttt gctggtcggt tgtctgaaaa gaattggggc 4860

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cct ggc att act act gcc att gag cag gct gct ctg gct gcg gcc aat   48
Pro Gly Ile Thr Thr Ala Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
  1               5                  10                  15

tct gcc ttg gcg aat gct gtg gtg gtt cgg ccg ttt tta tct cgc gtg   96
Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val
            20                  25                  30

caa acc gag att ctt att aat ttg atg caa ccc cgg cag ttg gtt ttc   144
Gln Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe
        35                  40                  45

cgc cct gag gta ctt tgg aat cac cct atc cag cgg gtt ata cat aat   192
Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
    50                  55                  60

gaa tta gaa cag tac tgc cgg gct cgg gct ggt cgt tgc ttg gag gtt   240
Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val
65                  70                  75                  80

gga gct cac cca aga tcc att aat gac aac ccc aac gtt ctg cat cgg   288
Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg
                85                  90                  95

tgt ttc ctt aga ccg gtt ggc cga gat gtt cag cgc tgg tac tct gcc   336
Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
            100                 105                 110

ccc acc cgc ggc cct gcg gct aat tgc cgc cgc tcc gcg ttg cgt ggt   384
Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
        115                 120                 125

ctc ccc ccc gct gac cgc act tac tgc ttt gat gga ttc tcc cgt tgt   432
Leu Pro Pro Ala Asp Arg Thr Tyr Cys Phe Asp Gly Phe Ser Arg Cys
    130                 135                 140

gct ttt gct gca gag acc ggt gtg gct ctt tac tct ctg cat gac ctt   480
Ala Phe Ala Ala Glu Thr Gly Val Ala Leu Tyr Ser Leu His Asp Leu
145                 150                 155                 160

tgg cca gct gat gtt gca gag gct atg gcc cgc cac ggg atr aca cgc   528
Trp Pro Ala Asp Val Ala Glu Ala Met Ala Arg His Gly Xaa Thr Arg
                165                 170                 175

ttg tat gcc gca ctg cac ctt ccc cct gag gtg ctg cta cca ccc ggc   576
Leu Tyr Ala Ala Leu His Leu Pro Pro Glu Val Leu Leu Pro Pro Gly
            180                 185                 190

acc tac cac aca acc tcg tat ctc ctg att cac gac ggc gac cgc gct   624
Thr Tyr His Thr Thr Ser Tyr Leu Leu Ile His Asp Gly Asp Arg Ala
        195                 200                 205

gtt gta act tac gag ggc gat act agt gcg ggc tat aat cat gat gtc   672
Val Val Thr Tyr Glu Gly Asp Thr Ser Ala Gly Tyr Asn His Asp Val
    210                 215                 220

tcc ata ctt cgt gcg tgg atc cgt act aca aaa ata gtt ggt gat cat   720
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Ser Ile Leu Arg Ala Trp Ile Arg Thr Thr Lys Ile Val Gly Asp His
225                 230                 235                 240

ccg ttg gtc ata gag cgt gtg cgg gcc att gga tgt cat ttt gtg ttg   768
Pro Leu Val Ile Glu Arg Val Arg Ala Ile Gly Cys His Phe Val Leu
                245                 250                 255

ctg ctc acc gca gcc cct gag ccg tca ccc atg cct tat gtt cct tac   816
Leu Leu Thr Ala Ala Pro Glu Pro Ser Pro Met Pro Tyr Val Pro Tyr
            260                 265                 270

cct cgt tca acg gag gtg tat gtc cgg tcc ata ttt ggc cct ggc ggc   864
Pro Arg Ser Thr Glu Val Tyr Val Arg Ser Ile Phe Gly Pro Gly Gly
        275                 280                 285

tcc cca tcc ttg ttt ccg tca gcc tgc tct act aaa tct act ttc cat   912
Ser Pro Ser Leu Phe Pro Ser Ala Cys Ser Thr Lys Ser Thr Phe His
    290                 295                 300

gct gtc ccg gtg cat atc tgg gat cgg ctc atg ctc ttt ggt gcc acc   960
Ala Val Pro Val His Ile Trp Asp Arg Leu Met Leu Phe Gly Ala Thr
305                 310                 315                 320

ctg gac gat cag gcg ttt tgc tgt tca cgg ctc atg act tac ctc cgt   1008
Leu Asp Asp Gln Ala Phe Cys Cys Ser Arg Leu Met Thr Tyr Leu Arg
                325                 330                 335

ggt att agt tac aag gtc act gtc ggc gcg ctt gtc gct aat gag ggg   1056
Gly Ile Ser Tyr Lys Val Thr Val Gly Ala Leu Val Ala Asn Glu Gly
            340                 345                 350

tgg aac gcc tct gaa gac gct ctt act gca rtg atc act gca gct tat   1104
Trp Asn Ala Ser Glu Asp Ala Leu Thr Ala Xaa Ile Thr Ala Ala Tyr
        355                 360                 365

ttg act att tgc cat cag cgt tat ctc cgc acc cag gcg ata tcc aag   1152
Leu Thr Ile Cys His Gln Arg Tyr Leu Arg Thr Gln Ala Ile Ser Lys
    370                 375                 380

ggc atg cgc cgg ttg ggg gtt gag cac gcc cag aaa ttt atc aca aga   1200
Gly Met Arg Arg Leu Gly Val Glu His Ala Gln Lys Phe Ile Thr Arg
385                 390                 395                 400

ctc tac agt tgg cta ttt gag aag tct ggc cgt gat tat atc ccc ggc   1248
Leu Tyr Ser Trp Leu Phe Glu Lys Ser Gly Arg Asp Tyr Ile Pro Gly
                405                 410                 415

cgc cag ctt cag ttc tat gca cag tgc cga cgg tgg cta tct gca ggc   1296
Arg Gln Leu Gln Phe Tyr Ala Gln Cys Arg Arg Trp Leu Ser Ala Gly
            420                 425                 430

ttc cac cta gac ccc agg gta ctt gtt ttt gat gag tca gta cca tgc   1344
Phe His Leu Asp Pro Arg Val Leu Val Phe Asp Glu Ser Val Pro Cys
        435                 440                 445

cgc tgt agg acg ttt ttg aag aaa gtt gcg ggt aaa ttc tgc tgt ttt   1392
Arg Cys Arg Thr Phe Leu Lys Lys Val Ala Gly Lys Phe Cys Cys Phe
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    450                 455                 460

atg cgg tgg ctc ggg cag gag tgt acc tgc ttc ttg gag ccg gcc gag   1440
Met Arg Trp Leu Gly Gln Glu Cys Thr Cys Phe Leu Glu Pro Ala Glu
465                 470                 475                 480

ggt tta gtc ggc gat cat ggc cat gac aac gag gcc tat gag ggt tct   1488
Gly Leu Val Gly Asp His Gly His Asp Asn Glu Ala Tyr Glu Gly Ser
                485                 490                 495

gag gtc gac ccg gct gaa cct gca cat ctt gat gtt tct ggg act tac   1536
Glu Val Asp Pro Ala Glu Pro Ala His Leu Asp Val Ser Gly Thr Tyr
            500                 505                 510

gcc gtc cac ggg cac cag ctt gag gcc ctc tat agg gca ctt aat gtc   1584
Ala Val His Gly His Gln Leu Glu Ala Leu Tyr Arg Ala Leu Asn Val
        515                 520                 525

cca caa gat att gcc gct cga gct tcc cga cta acg gca act gtt gag   1632
Pro Gln Asp Ile Ala Ala Arg Ala Ser Arg Leu Thr Ala Thr Val Glu
    530                 535                 540

ctc gtt gca agt cca gac cgc tta gag tgc cgc acc gtg ctc ggt aat   1680
Leu Val Ala Ser Pro Asp Arg Leu Glu Cys Arg Thr Val Leu Gly Asn
545                 550                 555                 560

aag acc ttc cgg acg acg gtg gtc gac ggc gcc cat cta gag gcg aat   1728
Lys Thr Phe Arg Thr Thr Val Val Asp Gly Ala His Leu Glu Ala Asn
                565                 570                 575

ggc cct gag cag tat gtc tta tca ttt gac gcc tcc cgt cag tct atg   1776
Gly Pro Glu Gln Tyr Val Leu Ser Phe Asp Ala Ser Arg Gln Ser Met
            580                 585                 590

ggg gcc ggg tcg cat agc ctc act tat gag ctc acc cct gct ggt ttg   1824
Gly Ala Gly Ser His Ser Leu Thr Tyr Glu Leu Thr Pro Ala Gly Leu
        595                 600                 605

cag gtt agg att tca tct aat ggt ctg gat tgc act gct aca ttc ccc   1872
Gln Val Arg Ile Ser Ser Asn Gly Leu Asp Cys Thr Ala Thr Phe Pro
    610                 615                 620

ccc ggt gga gcc cct agc gct gcg ccc ggg gag gtg gca gcc ttt tgc   1920
Pro Gly Gly Ala Pro Ser Ala Ala Pro Gly Glu Val Ala Ala Phe Cys
625                 630                 635                 640

agt gcc ctt tat aga tat aac agg ttc acc cag cgg cac tcg ctg act   1968
Ser Ala Leu Tyr Arg Tyr Asn Arg Phe Thr Gln Arg His Ser Leu Thr
                645                 650                 655

ggc gga tta tgg tta cac cct gag ggg ttg ctg ggt att ttc ccc cct   2016
Gly Gly Leu Trp Leu His Pro Glu Gly Leu Leu Gly Ile Phe Pro Pro
            660                 665                 670

ttc tcc cct ggg cat atc tgg gag tct gcg aac ccc ttt tgc ggg gag   2064
Phe Ser Pro Gly His Ile Trp Glu Ser Ala Asn Pro Phe Cys Gly Glu
        675                 680                 685
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ggg act ttg tat acc cga act tgg tca aca tct ggc ttt tct agt gat   2112
Gly Thr Leu Tyr Thr Arg Thr Trp Ser Thr Ser Gly Phe Ser Ser Asp
    690                 695                 700

ttc tcc ccc cct gaa gcg gcc gct cct gct atg gct gct acc ccg ggg   2160
Phe Ser Pro Pro Glu Ala Ala Ala Pro Ala Met Ala Ala Thr Pro Gly
705                 710                 715                 720

ctg ccc cat tct acc cca cct gtt agc gat att tgg gtg cta cca ccg   2208
Leu Pro His Ser Thr Pro Pro Val Ser Asp Ile Trp Val Leu Pro Pro
                725                 730                 735

ccc tca gag gag ttt cag gtt gat gca gca cct gtg ccc cct gcc cct   2256
Pro Ser Glu Glu Phe Gln Val Asp Ala Ala Pro Val Pro Pro Ala Pro
            740                 745                 750

gac cct gct gga ttg ccc ggt ccc gtt gtg ctt acc ccc ccc ccc cct   2304
Asp Pro Ala Gly Leu Pro Gly Pro Val Val Leu Thr Pro Pro Pro Pro
        755                 760                 765

ccc cct gtg cat aag cca tca ata ccc ccg cct tcc cgt aac cgt cgt   2352
Pro Pro Val His Lys Pro Ser Ile Pro Pro Pro Ser Arg Asn Arg Arg
    770                 775                 780

ctc ctc tat acc tat cct gac ggc gct aag gtg tat gca ggg tca ctg   2400
Leu Leu Tyr Thr Tyr Pro Asp Gly Ala Lys Val Tyr Ala Gly Ser Leu
785                 790                 795                 800

ttt gaa tca gac tgt gac tgg ctg gtt aat gcc tca aac ccg ggc cat   2448
Phe Glu Ser Asp Cys Asp Trp Leu Val Asn Ala Ser Asn Pro Gly His
                805                 810                 815

cgt ccc gga ggt ggc ctc tgc cat gcc ttt tac caa cgt ttt cca gaa   2496
Arg Pro Gly Gly Gly Leu Cys His Ala Phe Tyr Gln Arg Phe Pro Glu
            820                 825                 830

gcg ttt tac cca act gaa ttc atc atg cgt gag ggt ctt gca gca tac   2544
Ala Phe Tyr Pro Thr Glu Phe Ile Met Arg Glu Gly Leu Ala Ala Tyr
        835                 840                 845

acc ttg acc ccg cgc cct atc att cat gca gtc gct ccc gat tat agg   2592
Thr Leu Thr Pro Arg Pro Ile Ile His Ala Val Ala Pro Asp Tyr Arg
    850                 855                 860

gtt gag cag aac ccg aag agg ctt gag gca gcg tac cgt gaa act tgt   2640
Val Glu Gln Asn Pro Lys Arg Leu Glu Ala Ala Tyr Arg Glu Thr Cys
865                 870                 875                 880

tcc cgt cgt ggc acc gct gcc tac ccg ctt ttg ggt tcg ggt ata tac   2688
Ser Arg Arg Gly Thr Ala Ala Tyr Pro Leu Leu Gly Ser Gly Ile Tyr
                885                 890                 895

cag gtc cct gtt agc ctc agt ttt gat gcc tgg gaa cgt aat cac cgc   2736
Gln Val Pro Val Ser Leu Ser Phe Asp Ala Trp Glu Arg Asn His Arg
            900                 905                 910
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ccc ggc gat gag ctt tac ttg acc gag ccc gct gca aat tgg ttt gag   2784
Pro Gly Asp Glu Leu Tyr Leu Thr Glu Pro Ala Ala Asn Trp Phe Glu
        915                 920                 925

gct aat aag ccg gcg cag ccg gtg ctc acc ata act gag gac acg gcc   2832
Ala Asn Lys Pro Ala Gln Pro Val Leu Thr Ile Thr Glu Asp Thr Ala
    930                 935                 940

cgt acg gcc aac ctg gca ttg gag att gat gcc gct aca gag gtc ggc   2880
Arg Thr Ala Asn Leu Ala Leu Glu Ile Asp Ala Ala Thr Glu Val Gly
945                 950                 955                 960

cgt gct tgt gcc ggt tgc acc atc agc cct ggc att gtg cac tat cag   2928
Arg Ala Cys Ala Gly Cys Thr Ile Ser Pro Gly Ile Val His Tyr Gln
                965                 970                 975

ttt acc gcc ggg gtc ccg ggc tcg ggc aag tca agg tcc ata caa cag   2976
Phe Thr Ala Gly Val Pro Gly Ser Gly Lys Ser Arg Ser Ile Gln Gln
            980                 985                 990

gga gat gtc gat gtg gtg gtt gtg ccc acc cgg gag ctt cgt aat agt   3024
Gly Asp Val Asp Val Val Val Val Pro Thr Arg Glu Leu Arg Asn Ser
        995                1000                1005

tgg cgc cgc cgg ggt ttt gcg gcc ttc aca ccc cac aca gcg gcc cgt   3072
Trp Arg Arg Arg Gly Phe Ala Ala Phe Thr Pro His Thr Ala Ala Arg
   1010                1015                1020

gtt act atc ggc cgc cgc gtt gtg att gat gag gct cca tct ctc ccg   3120
Val Thr Ile Gly Arg Arg Val Val Ile Asp Glu Ala Pro Ser Leu Pro
1025                1030                1035                1040

cca cac ctg ttg ctg tta cat atg cag cgg gcc tcc tcg gtc cat ctc   3168
Pro His Leu Leu Leu Leu His Met Gln Arg Ala Ser Ser Val His Leu
                1045                1050                1055

ctc ggt gac cca aat cag atc cct gct att gat ttt gag cac gcc ggc   3216
Leu Gly Asp Pro Asn Gln Ile Pro Ala Ile Asp Phe Glu His Ala Gly
            1060                1065                1070

ctg gtc cct gcg atc cgt ccc gag ctt gcg cca acg agc tgg tgg crc   3264
Leu Val Pro Ala Ile Arg Pro Glu Leu Ala Pro Thr Ser Trp Trp Xaa
        1075                1080                1085

gtt aca cac cgt tgc ccg gcc gat gtg tgc gag ctc ata cgc gga gcc   3312
Val Thr His Arg Cys Pro Ala Asp Val Cys Glu Leu Ile Arg Gly Ala
   1090                1095                1100

tac cct aaa atc cag acc acg agc cgt gtg cta cgg tcc ctg ttt tgg   3360
Tyr Pro Lys Ile Gln Thr Thr Ser Arg Val Leu Arg Ser Leu Phe Trp
1105                1110                1115                1120

aat gaa ccg gcc att ggc cag aag ttg gtt ytc acg cag gcg gca aag   3408
Asn Glu Pro Ala Ile Gly Gln Lys Leu Val Xaa Thr Gln Ala Ala Lys
                1125                1130                1135

gct gct aac cct ggt gcg att acg gtc cac gaa gct cag ggt gcc acc   3456
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Ala Ala Asn Pro Gly Ala Ile Thr Val His Glu Ala Gln Gly Ala Thr
          1140                1145                1150

ttc aca gag acc aca atc ata gcc acg gcc gac gcc agg ggc ctt atc   3504
Phe Thr Glu Thr Thr Ile Ile Ala Thr Ala Asp Ala Arg Gly Leu Ile
       1155                1160                1165

cag tca tcc cgg gct cat gct ata gtt gca ctt act cgc cac act gag   3552
Gln Ser Ser Arg Ala His Ala Ile Val Ala Leu Thr Arg His Thr Glu
   1170                1175                1180

aag tgt gtt atc ctg gat gcc ccc ggc ctg ctt cgt gag gtc ggc att   3600
Lys Cys Val Ile Leu Asp Ala Pro Gly Leu Leu Arg Glu Val Gly Ile
1185                1190                1195                1200

tcg gat gtg att gtc aac aac ttt ttc ctt gct ggt ggc gag gtc ggc   3648
Ser Asp Val Ile Val Asn Asn Phe Phe Leu Ala Gly Gly Glu Val Gly
               1205                1210                1215

crc cac cgc cct tct gtg ata cct cgc ggt aac cct gat caa aac ctc   3696
Xaa His Arg Pro Ser Val Ile Pro Arg Gly Asn Pro Asp Gln Asn Leu
          1220                1225                1230

ggg act tta cag gcc ttc ccg ccg tcc tgt caa att agt gct tac cat   3744
Gly Thr Leu Gln Ala Phe Pro Pro Ser Cys Gln Ile Ser Ala Tyr His
       1235                1240                1245

cag ttg gct gag gaa ctg ggc cat cgc ccg gcc cct gtc gcc gcc gtc   3792
Gln Leu Ala Glu Glu Leu Gly His Arg Pro Ala Pro Val Ala Ala Val
   1250                1255                1260

ttg ccc cct tgc cct gag ctt gag cag ggc ctg ctc tac atg cca cag   3840
Leu Pro Pro Cys Pro Glu Leu Glu Gln Gly Leu Leu Tyr Met Pro Gln
1265                1270                1275                1280

gag ctc act gtg tcc gat agt gtg ttg gtt ttt gag ctt acg gat ata   3888
Glu Leu Thr Val Ser Asp Ser Val Leu Val Phe Glu Leu Thr Asp Ile
               1285                1290                1295

gtt cat tgc cgc atg gcc gct cca agc cag cga aag gct gtt ctc tca   3936
Val His Cys Arg Met Ala Ala Pro Ser Gln Arg Lys Ala Val Leu Ser
          1300                1305                1310

aca ctt gtg ggg agg tat ggc cgt agg acg aaa cta tat gag gcg gcg   3984
Thr Leu Val Gly Arg Tyr Gly Arg Arg Thr Lys Leu Tyr Glu Ala Ala
       1315                1320                1325

cat tca gat gtt cgt gag tcc cta gct agg ttc atc cct act atc ggg   4032
His Ser Asp Val Arg Glu Ser Leu Ala Arg Phe Ile Pro Thr Ile Gly
   1330                1335                1340

cct gtt cag gct acc aca tgt gag ttg tat gag ttg gtt gag gct atg   4080
Pro Val Gln Ala Thr Thr Cys Glu Leu Tyr Glu Leu Val Glu Ala Met
1345                1350                1355                1360

gtg gag aaa ggt cag gac ggc tct gca gtc tta gag ctt gat ctt tgt   4128
Val Glu Lys Gly Gln Asp Gly Ser Ala Val Leu Glu Leu Asp Leu Cys
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                1365                1370                1375

aat cgt gat gtc tcg cgc atc aca ttt ttc caa aaa gwc tgc aac aag   4176
Asn Arg Asp Val Ser Arg Ile Thr Phe Phe Gln Lys Xaa Cys Asn Lys
           1380                1385                1390

ttt aca act ggt gag acc atc gcc cac ggc aag gtt ggc cag ggt ata   4224
Phe Thr Thr Gly Glu Thr Ile Ala His Gly Lys Val Gly Gln Gly Ile
       1395                1400                1405

tcg gcc tgg agt aag acc ttc tgc gct ctg ttc ggc ccg tgg ttc cgc   4272
Ser Ala Trp Ser Lys Thr Phe Cys Ala Leu Phe Gly Pro Trp Phe Arg
   1410                1415                1420

gcc att gaa aaa gaa ata ttg gcc ctg ctc ccg cct aat atc ttt tat   4320
Ala Ile Glu Lys Glu Ile Leu Ala Leu Leu Pro Pro Asn Ile Phe Tyr
1425                1430                1435                1440

ggc gac gct tat gag gag tca gtt ttt gcc gcc gct gtg tcc ggg gcg   4368
Gly Asp Ala Tyr Glu Glu Ser Val Phe Ala Ala Ala Val Ser Gly Ala
               1445                1450                1455

ggg tca tgt atg gta ttt gaa aat gac ttt tca gag ttt gac agt acc   4416
Gly Ser Cys Met Val Phe Glu Asn Asp Phe Ser Glu Phe Asp Ser Thr
           1460                1465                1470

cag aat aat ttc tct ctt ggc ctt gag tgt gtg gtt atg gag gag tgc   4464
Gln Asn Asn Phe Ser Leu Gly Leu Glu Cys Val Val Met Glu Glu Cys
       1475                1480                1485

ggc atg cct caa tgg cta att agg ttg tac cat ctg gtt cgg tct gcc   4512
Gly Met Pro Gln Trp Leu Ile Arg Leu Tyr His Leu Val Arg Ser Ala
   1490                1495                1500

tgg att ctg cag gcg ccg aag gag tct ctt aag ggt ttc tgg aag aag   4560
Trp Ile Leu Gln Ala Pro Lys Glu Ser Leu Lys Gly Phe Trp Lys Lys
1505                1510                1515                1520

cat tct ggt gag cct ggt acc ctt ctt tgg aat acc gtc tgg aat atg   4608
His Ser Gly Glu Pro Gly Thr Leu Leu Trp Asn Thr Val Trp Asn Met
               1525                1530                1535

gcg att ata gca cat tgc tat gag ttc cgt gac ttt cgt gtt gct gcc   4656
Ala Ile Ile Ala His Cys Tyr Glu Phe Arg Asp Phe Arg Val Ala Ala
           1540                1545                1550

ttt aag ggt gat gat tcg gtg gtc ctc tgt agt gac tac cga cag agc   4704
Phe Lys Gly Asp Asp Ser Val Val Leu Cys Ser Asp Tyr Arg Gln Ser
       1555                1560                1565

cgc aat gca gct gcc tta att gct ggc tgt ggg ctc aaa ttg aag gtt   4752
Arg Asn Ala Ala Ala Leu Ile Ala Gly Cys Gly Leu Lys Leu Lys Val
   1570                1575                1580

gat tac cgc cct atc ggg ctg tat gct ggg gtg gtg gtg gcc ccc ggt   4800
Asp Tyr Arg Pro Ile Gly Leu Tyr Ala Gly Val Val Val Ala Pro Gly
1585                1590                1595                1600
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ttg ggg aca ctg ccc gat gtg gtg cgt ttt gct ggt cgg ttg tct gaa   4848
Leu Gly Thr Leu Pro Asp Val Val Arg Phe Ala Gly Arg Leu Ser Glu
                1605                1610                1615

aag aat tgg ggc ccc ggc ccg gaa cgt gct gag cag ctg cgt ctt gct   4896
Lys Asn Trp Gly Pro Gly Pro Glu Arg Ala Glu Gln Leu Arg Leu Ala
            1620                1625                1630

gtc tgc gac ttc ctt cga ggg ttg acg aat gtt gcg cag gtc tgt gtt   4944
Val Cys Asp Phe Leu Arg Gly Leu Thr Asn Val Ala Gln Val Cys Val
        1635                1640                1645

gat gtt gtg tcc cgt gtc tat gga gtc agc ccc ggg ctc gta cat aac   4992
Asp Val Val Ser Arg Val Tyr Gly Val Ser Pro Gly Leu Val His Asn
    1650                1655                1660

ctt att ggc atg ctg cag acc atc gcc gat ggc aag gcc cac ttt aca   5040
Leu Ile Gly Met Leu Gln Thr Ile Ala Asp Gly Lys Ala His Phe Thr
1665                1670                1675                1680

gag act att aaa cct gta ctt gat ctc aca aat tcc atc ata cag cgg   5088
Glu Thr Ile Lys Pro Val Leu Asp Leu Thr Asn Ser Ile Ile Gln Arg
                1685                1690                1695

gtg gaa tga ataacatgtc ttttgcatcg cccatgggat cacc atg cgc cct agg 5143
Val Glu                                          Met Arg Pro Arg
                                                 1700

gct gtt ctg ttg ttg ttc ctc atg ttt ctg cct atg ctg ccc gcg cca   5191
Ala Val Leu Leu Leu Phe Leu Met Phe Leu Pro Met Leu Pro Ala Pro
    1705                1710                1715

ccg gcc ggt cag ccg tct ggc cgt cgc cgt ggg cgg cgc agc ggc ggt   5239
Pro Ala Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg Arg Ser Gly Gly
1720                1725                1730                1735

gcc ggc ggt ggt ttc tgg agt gac agg gtt gat tct cag ccc ttc gcc   5287
Ala Gly Gly Gly Phe Trp Ser Asp Arg Val Asp Ser Gln Pro Phe Ala
                1740                1745                1750

ctc ccc tat att cat cca acc aac ccc ttc gcc gcc gat gtc gtt tca   5335
Leu Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Ala Asp Val Val Ser
            1755                1760                1765

caa ccc ggg gct gga act cgc cct cga cag ccg ccc cgc ccc ctc ggt   5383
Gln Pro Gly Ala Gly Thr Arg Pro Arg Gln Pro Pro Arg Pro Leu Gly
        1770                1775                1780

tcc gct tgg cgt gac cag tcc aag cgc ccc tcc gtt gcc ccc cgt cgt   5431
Ser Ala Trp Arg Asp Gln Ser Lys Arg Pro Ser Val Ala Pro Arg Arg
    1785                1790                1795

cga tct acc cca gct ggg gct gcg ccg cta act gcc ata tca cca gcc   5479
Arg Ser Thr Pro Ala Gly Ala Ala Pro Leu Thr Ala Ile Ser Pro Ala
1800                1805                1810                1815
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cct gat aca gct cct gta cct gat gtt gac tca cgt ggt gct att ttg   5527
Pro Asp Thr Ala Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu
               1820                1825                1830

cgc cgg cag tac aat ttg tct acg tcc ccg ctt aca tca tct gtt gct   5575
Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr Ser Ser Val Ala
           1835                1840                1845

tct ggt act aat ctg gtt ctc tat gct gcc ccg ctg aac cct ctc ttg   5623
Ser Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Asn Pro Leu Leu
       1850                1855                1860

cct ctt cag gat ggc acc aac act cat att atg gct act gag gca tct   5671
Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu Ala Ser
   1865                1870                1875

aat tac gcc cag tat cgg gtt gtt cgg gct acg att cgt tat cgc ccg   5719
Asn Tyr Ala Gln Tyr Arg Val Val Arg Ala Thr Ile Arg Tyr Arg Pro
1880                1885                1890                1895

ttg gtg cca aat gct gtt ggt ggt tat gct atc tct att tct ttc tgg   5767
Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile Ser Phe Trp
               1900                1905                1910

cct caa act aca act acc cct act tct gtt gac atg aat tct atc act   5815
Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser Ile Thr
           1915                1920                1925

tct act gat gtc agg atc ttg gtc cag ccc ggt ata gcc tcc gag tta   5863
Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser Glu Leu
       1930                1935                1940

gtc atc cct agt gaa cgc ctt cac tac cgc aac caa ggc tgg cgc tct   5911
Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp Arg Ser
   1945                1950                1955

gtt gag acc acg ggt gtg gcc gaa gag gag gct acc tcc ggt ctg gta   5959
Val Glu Thr Thr Gly Val Ala Glu Glu Glu Ala Thr Ser Gly Leu Val
1960                1965                1970                1975

atg ctt tgt att cat ggc tcc cct gtt aac tcc tac act aat aca cct   6007
Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn Thr Pro
               1980                1985                1990

tac acc ggt gca ttg ggg ctt ctt gat ttt gca tta gaa ctt gaa ttt   6055
Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu Glu Phe
           1995                2000                2005

aga aat ttg aca ccc ggg aac act aac acc cgt gtt tcc cgg tat act   6103
Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser Arg Tyr Thr
       2010                2015                2020

agc aca gcc cgc cac cgg ctg cgc cgc ggt gct gat ggg acc gct gag   6151
Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp Gly Thr Ala Glu
   2025                2030                2035

ctc acc acc aca gca gcc aca cgc ttc atg aag gat ttg cat ttt act   6199
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Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu His Phe Thr
2040                2045                2050                2055

ggt acg aac ggc gtt ggt gag gtg ggt cgt ggt att gcc ctg act ctg   6247
Gly Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile Ala Leu Thr Leu
                2060                2065                2070

ttt aat ctt gct gat acg ctt ctt ggt ggt tta ccg aca gaa ttg att   6295
Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr Glu Leu Ile
            2075                2080                2085

tcg tcg gct ggg ggt caa ctg ttt tac tcc cgc cct gtt gtc tcg gcc   6343
Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val Val Ser Ala
        2090                2095                2100

aat ggc gag cca aca gta aag tta tac aca tct gtt gag aat gcg cag   6391
Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln
    2105                2110                2115

caa gac aag ggc atc acc att cca cac gac ata gat tta ggt gac tcc   6439
Gln Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp Leu Gly Asp Ser
2120                2125                2130                2135

cgt gtg gtt atc cag gat tat gat aac cag cac gaa caa gat cga cct   6487
Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln Asp Arg Pro
                2140                2145                2150

acc ccg tca cct gcc ccc tcc cgc cct ttc tca gtt ctt cgt gcc aat   6535
Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu Arg Ala Asn
            2155                2160                2165

gat gtt ttg tgg ctc tct ctc act gcc gct gag tac grc cag acc acg   6583
Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Xaa Gln Thr Thr
        2170                2175                2180

tat ggg tcg tcc acc aac cct atg tat gtc tct gat aca gtc acg ctt   6631
Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp Thr Val Thr Leu
    2185                2190                2195

gtt aat gta gcc act ggt gct cag gct gtt gcc cgc tct ctt gac tgg   6679
Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser Leu Asp Trp
2200                2205                2210                2215

tct aaa gtt act ctg gat ggt cgc cct ctt act acc att cag cag tat   6727
Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr Ile Gln Gln Tyr
                2220                2225                2230

tct aag aaa ttt tat gtt ctc ccg ctt cgs ggg aag ctg tcc ttt tgg   6775
Ser Lys Lys Phe Tyr Val Leu Pro Leu Xaa Gly Lys Leu Ser Phe Trp
            2235                2240                2245

gag gct ggt acg acc aag gcc ggc tac ccg tat aat tat aat acc act   6823
Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr
        2250                2255                2260

gct agt gac caa att ttg att gag aac gcg gcc ggt cac cgt gtc gcc   6871
Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly His Arg Val Ala
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   2265                2270                2275

att tct act tat acc act agt ttg ggt gcc ggc cct acc tcg aty tct   6919
Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Thr Ser Xaa Ser
2280                2285                2290                2295

gcg gtc ggt gta cta gct cca cat tcg gcc ctt gct gtt ctc gag gat   6967
Ala Val Gly Val Leu Ala Pro His Ser Ala Leu Ala Val Leu Glu Asp
               2300                2305                2310

act gtt gat tat cct gct cgt gcc cat act ttt gat gat ttc tgc ccg   7015
Thr Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro
           2315                2320                2325

gag tgt cgc acc ctt ggt ctg cag ggt tgt gca ttc caa tct act att   7063
Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Ile
       2330                2335                2340

gct gaa ctt cag cgt ctt aaa atg aag gta ggt aaa acc cgg gag tct   7111
Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu Ser
   2345                2350                2355

taa ttaattcctt ttgtgccccc ttcgcagttc tctttggctt tatttctcat        7164

2360

ttctgctttc cgcgctccct ggaaaaaaaa aaaaaaaa                        7202


<210> 91
<211> 1698
<212> PRT
<213> Hepatitis E virus

<400> 91
Pro Gly Ile Thr Thr Ala Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
  1               5                  10                  15

Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val
             20                  25                  30

Gln Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe
         35                  40                  45

Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
     50                  55                  60

Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val
 65                  70                  75                  80

Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg
                 85                  90                  95

Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
            100                 105                 110

Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
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        115                 120                 125

Leu Pro Pro Ala Asp Arg Thr Tyr Cys Phe Asp Gly Phe Ser Arg Cys
    130                 135                 140

Ala Phe Ala Ala Glu Thr Gly Val Ala Leu Tyr Ser Leu His Asp Leu
145                 150                 155                 160

Trp Pro Ala Asp Val Ala Glu Ala Met Ala Arg His Gly Xaa Thr Arg
                165                 170                 175

Leu Tyr Ala Ala Leu His Leu Pro Pro Glu Val Leu Leu Pro Pro Gly
            180                 185                 190

Thr Tyr His Thr Thr Ser Tyr Leu Leu Ile His Asp Gly Asp Arg Ala
        195                 200                 205

Val Val Thr Tyr Glu Gly Asp Thr Ser Ala Gly Tyr Asn His Asp Val
    210                 215                 220

Ser Ile Leu Arg Ala Trp Ile Arg Thr Thr Lys Ile Val Gly Asp His
225                 230                 235                 240

Pro Leu Val Ile Glu Arg Val Arg Ala Ile Gly Cys His Phe Val Leu
                245                 250                 255

Leu Leu Thr Ala Ala Pro Glu Pro Ser Pro Met Pro Tyr Val Pro Tyr
            260                 265                 270

Pro Arg Ser Thr Glu Val Tyr Val Arg Ser Ile Phe Gly Pro Gly Gly
        275                 280                 285

Ser Pro Ser Leu Phe Pro Ser Ala Cys Ser Thr Lys Ser Thr Phe His
    290                 295                 300

Ala Val Pro Val His Ile Trp Asp Arg Leu Met Leu Phe Gly Ala Thr
305                 310                 315                 320

Leu Asp Asp Gln Ala Phe Cys Cys Ser Arg Leu Met Thr Tyr Leu Arg
                325                 330                 335

Gly Ile Ser Tyr Lys Val Thr Val Gly Ala Leu Val Ala Asn Glu Gly
            340                 345                 350

Trp Asn Ala Ser Glu Asp Ala Leu Thr Ala Xaa Ile Thr Ala Ala Tyr
        355                 360                 365

Leu Thr Ile Cys His Gln Arg Tyr Leu Arg Thr Gln Ala Ile Ser Lys
    370                 375                 380

Gly Met Arg Arg Leu Gly Val Glu His Ala Gln Lys Phe Ile Thr Arg
385                 390                 395                 400

Leu Tyr Ser Trp Leu Phe Glu Lys Ser Gly Arg Asp Tyr Ile Pro Gly
                405                 410                 415

Arg Gln Leu Gln Phe Tyr Ala Gln Cys Arg Arg Trp Leu Ser Ala Gly
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```
            420                 425                 430

Phe His Leu Asp Pro Arg Val Leu Val Phe Asp Glu Ser Val Pro Cys
        435                 440                 445

Arg Cys Arg Thr Phe Leu Lys Lys Val Ala Gly Lys Phe Cys Cys Phe
    450                 455                 460

Met Arg Trp Leu Gly Gln Glu Cys Thr Cys Phe Leu Glu Pro Ala Glu
465                 470                 475                 480

Gly Leu Val Gly Asp His Gly His Asp Asn Glu Ala Tyr Glu Gly Ser
                485                 490                 495

Glu Val Asp Pro Ala Glu Pro Ala His Leu Asp Val Ser Gly Thr Tyr
            500                 505                 510

Ala Val His Gly His Gln Leu Glu Ala Leu Tyr Arg Ala Leu Asn Val
        515                 520                 525

Pro Gln Asp Ile Ala Ala Arg Ala Ser Arg Leu Thr Ala Thr Val Glu
    530                 535                 540

Leu Val Ala Ser Pro Asp Arg Leu Glu Cys Arg Thr Val Leu Gly Asn
545                 550                 555                 560

Lys Thr Phe Arg Thr Thr Val Val Asp Gly Ala His Leu Glu Ala Asn
                565                 570                 575

Gly Pro Glu Gln Tyr Val Leu Ser Phe Asp Ala Ser Arg Gln Ser Met
            580                 585                 590

Gly Ala Gly Ser His Ser Leu Thr Tyr Glu Leu Thr Pro Ala Gly Leu
        595                 600                 605

Gln Val Arg Ile Ser Ser Asn Gly Leu Asp Cys Thr Ala Thr Phe Pro
    610                 615                 620

Pro Gly Gly Ala Pro Ser Ala Ala Pro Gly Glu Val Ala Ala Phe Cys
625                 630                 635                 640

Ser Ala Leu Tyr Arg Tyr Asn Arg Phe Thr Gln Arg His Ser Leu Thr
                645                 650                 655

Gly Gly Leu Trp Leu His Pro Glu Gly Leu Leu Gly Ile Phe Pro Pro
            660                 665                 670

Phe Ser Pro Gly His Ile Trp Glu Ser Ala Asn Pro Phe Cys Gly Glu
        675                 680                 685

Gly Thr Leu Tyr Thr Arg Thr Trp Ser Thr Ser Gly Phe Ser Ser Asp
    690                 695                 700

Phe Ser Pro Pro Glu Ala Ala Ala Pro Ala Met Ala Ala Thr Pro Gly
705                 710                 715                 720

Leu Pro His Ser Thr Pro Pro Val Ser Asp Ile Trp Val Leu Pro Pro
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                  725                 730                 735

Pro Ser Glu Glu Phe Gln Val Asp Ala Ala Pro Val Pro Pro Ala Pro
            740                 745                 750

Asp Pro Ala Gly Leu Pro Gly Pro Val Val Leu Thr Pro Pro Pro Pro
        755                 760                 765

Pro Pro Val His Lys Pro Ser Ile Pro Pro Pro Ser Arg Asn Arg Arg
    770                 775                 780

Leu Leu Tyr Thr Tyr Pro Asp Gly Ala Lys Val Tyr Ala Gly Ser Leu
785                 790                 795                 800

Phe Glu Ser Asp Cys Asp Trp Leu Val Asn Ala Ser Asn Pro Gly His
                805                 810                 815

Arg Pro Gly Gly Gly Leu Cys His Ala Phe Tyr Gln Arg Phe Pro Glu
            820                 825                 830

Ala Phe Tyr Pro Thr Glu Phe Ile Met Arg Glu Gly Leu Ala Ala Tyr
        835                 840                 845

Thr Leu Thr Pro Arg Pro Ile Ile His Ala Val Ala Pro Asp Tyr Arg
    850                 855                 860

Val Glu Gln Asn Pro Lys Arg Leu Glu Ala Ala Tyr Arg Glu Thr Cys
865                 870                 875                 880

Ser Arg Arg Gly Thr Ala Ala Tyr Pro Leu Leu Gly Ser Gly Ile Tyr
                885                 890                 895

Gln Val Pro Val Ser Leu Ser Phe Asp Ala Trp Glu Arg Asn His Arg
            900                 905                 910

Pro Gly Asp Glu Leu Tyr Leu Thr Glu Pro Ala Ala Asn Trp Phe Glu
        915                 920                 925

Ala Asn Lys Pro Ala Gln Pro Val Leu Thr Ile Thr Glu Asp Thr Ala
    930                 935                 940

Arg Thr Ala Asn Leu Ala Leu Glu Ile Asp Ala Ala Thr Glu Val Gly
945                 950                 955                 960

Arg Ala Cys Ala Gly Cys Thr Ile Ser Pro Gly Ile Val His Tyr Gln
                965                 970                 975

Phe Thr Ala Gly Val Pro Gly Ser Gly Lys Ser Arg Ser Ile Gln Gln
            980                 985                 990

Gly Asp Val Asp Val Val Val Val Pro Thr Arg Glu Leu Arg Asn Ser
        995                1000                1005

Trp Arg Arg Arg Gly Phe Ala Ala Phe Thr Pro His Thr Ala Ala Arg
   1010                1015                1020

Val Thr Ile Gly Arg Arg Val Val Ile Asp Glu Ala Pro Ser Leu Pro
```

025 1030 1035 1040

Pro His Leu Leu Leu Leu His Met Gln Arg Ala Ser Ser Val His Leu
1045 1050 1055

Leu Gly Asp Pro Asn Gln Ile Pro Ala Ile Asp Phe Glu His Ala Gly
1060 1065 1070

Leu Val Pro Ala Ile Arg Pro Glu Leu Ala Pro Thr Ser Trp Trp Xaa
1075 1080 1085

Val Thr His Arg Cys Pro Ala Asp Val Cys Glu Leu Ile Arg Gly Ala
1090 1095 1100

Tyr Pro Lys Ile Gln Thr Thr Ser Arg Val Leu Arg Ser Leu Phe Trp
105 1110 1115 1120

Asn Glu Pro Ala Ile Gly Gln Lys Leu Val Xaa Thr Gln Ala Ala Lys
1125 1130 1135

Ala Ala Asn Pro Gly Ala Ile Thr Val His Glu Ala Gln Gly Ala Thr
1140 1145 1150

Phe Thr Glu Thr Thr Ile Ile Ala Thr Ala Asp Ala Arg Gly Leu Ile
1155 1160 1165

Gln Ser Ser Arg Ala His Ala Ile Val Ala Leu Thr Arg His Thr Glu
1170 1175 1180

Lys Cys Val Ile Leu Asp Ala Pro Gly Leu Leu Arg Glu Val Gly Ile
185 1190 1195 1200

Ser Asp Val Ile Val Asn Asn Phe Phe Leu Ala Gly Gly Glu Val Gly
1205 1210 1215

Xaa His Arg Pro Ser Val Ile Pro Arg Gly Asn Pro Asp Gln Asn Leu
1220 1225 1230

Gly Thr Leu Gln Ala Phe Pro Pro Ser Cys Gln Ile Ser Ala Tyr His
1235 1240 1245

Gln Leu Ala Glu Glu Leu Gly His Arg Pro Ala Pro Val Ala Ala Val
1250 1255 1260

Leu Pro Pro Cys Pro Glu Leu Glu Gln Gly Leu Leu Tyr Met Pro Gln
265 1270 1275 1280

Glu Leu Thr Val Ser Asp Ser Val Leu Val Phe Glu Leu Thr Asp Ile
1285 1290 1295

Val His Cys Arg Met Ala Ala Pro Ser Gln Arg Lys Ala Val Leu Ser
1300 1305 1310

Thr Leu Val Gly Arg Tyr Gly Arg Arg Thr Lys Leu Tyr Glu Ala Ala
1315 1320 1325

His Ser Asp Val Arg Glu Ser Leu Ala Arg Phe Ile Pro Thr Ile Gly

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   1330                1335                1340

Pro Val Gln Ala Thr Thr Cys Glu Leu Tyr Glu Leu Val Glu Ala Met
345                1350                1355                1360

Val Glu Lys Gly Gln Asp Gly Ser Ala Val Leu Glu Leu Asp Leu Cys
               1365                1370                1375

Asn Arg Asp Val Ser Arg Ile Thr Phe Phe Gln Lys Xaa Cys Asn Lys
           1380                1385                1390

Phe Thr Thr Gly Glu Thr Ile Ala His Gly Lys Val Gly Gln Gly Ile
       1395                1400                1405

Ser Ala Trp Ser Lys Thr Phe Cys Ala Leu Phe Gly Pro Trp Phe Arg
   1410                1415                1420

Ala Ile Glu Lys Glu Ile Leu Ala Leu Leu Pro Pro Asn Ile Phe Tyr
425                1430                1435                1440

Gly Asp Ala Tyr Glu Glu Ser Val Phe Ala Ala Ala Val Ser Gly Ala
               1445                1450                1455

Gly Ser Cys Met Val Phe Glu Asn Asp Phe Ser Glu Phe Asp Ser Thr
           1460                1465                1470

Gln Asn Asn Phe Ser Leu Gly Leu Glu Cys Val Val Met Glu Glu Cys
       1475                1480                1485

Gly Met Pro Gln Trp Leu Ile Arg Leu Tyr His Leu Val Arg Ser Ala
   1490                1495                1500

Trp Ile Leu Gln Ala Pro Lys Glu Ser Leu Lys Gly Phe Trp Lys Lys
505                1510                1515                1520

His Ser Gly Glu Pro Gly Thr Leu Leu Trp Asn Thr Val Trp Asn Met
               1525                1530                1535

Ala Ile Ile Ala His Cys Tyr Glu Phe Arg Asp Phe Arg Val Ala Ala
           1540                1545                1550

Phe Lys Gly Asp Asp Ser Val Val Leu Cys Ser Asp Tyr Arg Gln Ser
       1555                1560                1565

Arg Asn Ala Ala Ala Leu Ile Ala Gly Cys Gly Leu Lys Leu Lys Val
   1570                1575                1580

Asp Tyr Arg Pro Ile Gly Leu Tyr Ala Gly Val Val Val Ala Pro Gly
585                1590                1595                1600

Leu Gly Thr Leu Pro Asp Val Val Arg Phe Ala Gly Arg Leu Ser Glu
               1605                1610                1615

Lys Asn Trp Gly Pro Gly Pro Glu Arg Ala Glu Gln Leu Arg Leu Ala
           1620                1625                1630

Val Cys Asp Phe Leu Arg Gly Leu Thr Asn Val Ala Gln Val Cys Val
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1635 1640 1645

Asp Val Val Ser Arg Val Tyr Gly Val Ser Pro Gly Leu Val His Asn
1650 1655 1660

Leu Ile Gly Met Leu Gln Thr Ile Ala Asp Gly Lys Ala His Phe Thr
665 1670 1675 1680

Glu Thr Ile Lys Pro Val Leu Asp Leu Thr Asn Ser Ile Ile Gln Arg
1685 1690 1695

Val Glu

<210> 92
<211> 660
<212> PRT
<213> Hepatitis E virus

<400> 92
Met Arg Pro Arg Ala Val Leu Leu Leu Phe Leu Met Phe
1 5 10

Leu Pro Met Leu Pro Ala Pro Pro Ala Gly Gln Pro Ser Gly Arg Arg
15 20 25

Arg Gly Arg Arg Ser Gly Gly Ala Gly Gly Gly Phe Trp Ser Asp Arg
30 35 40 45

Val Asp Ser Gln Pro Phe Ala Leu Pro Tyr Ile His Pro Thr Asn Pro
50 55 60

Phe Ala Ala Asp Val Val Ser Gln Pro Gly Ala Gly Thr Arg Pro Arg
65 70 75

Gln Pro Pro Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ser Lys Arg
80 85 90

Pro Ser Val Ala Pro Arg Arg Arg Ser Thr Pro Ala Gly Ala Ala Pro
95 100 105

Leu Thr Ala Ile Ser Pro Ala Pro Asp Thr Ala Pro Val Pro Asp Val
10 115 120 125

Asp Ser Arg Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser
130 135 140

Pro Leu Thr Ser Ser Val Ala Ser Gly Thr Asn Leu Val Leu Tyr Ala
145 150 155

Ala Pro Leu Asn Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His
160 165 170

Ile Met Ala Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Val Arg
175 180 185

Ala Thr Ile Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr

90 195 200 205

Ala Ile Ser Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser
210 215 220

Val Asp Met Asn Ser Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln
225 230 235

Pro Gly Ile Ala Ser Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr
240 245 250

Arg Asn Gln Gly Trp Arg Ser Val Glu Thr Thr Gly Val Ala Glu Glu
255 260 265

Glu Ala Thr Ser Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val
70 275 280 285

Asn Ser Tyr Thr Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp
290 295 300

Phe Ala Leu Glu Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn
305 310 315

Thr Arg Val Ser Arg Tyr Thr Ser Thr Ala Arg His Arg Leu Arg Arg
320 325 330

Gly Ala Asp Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe
335 340 345

Met Lys Asp Leu His Phe Thr Gly Thr Asn Gly Val Gly Glu Val Gly
50 355 360 365

Arg Gly Ile Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly
370 375 380

Gly Leu Pro Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr
385 390 395

Ser Arg Pro Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr
400 405 410

Thr Ser Val Glu Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His
415 420 425

Asp Ile Asp Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn
30 435 440 445

Gln His Glu Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro
450 455 460

Phe Ser Val Leu Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala
465 470 475

Ala Glu Tyr Xaa Gln Thr Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr
480 485 490

Val Ser Asp Thr Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala

```
   495                 500                 505

Val Ala Arg Ser Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro
10                 515                 520                 525

Leu Thr Thr Ile Gln Gln Tyr Ser Lys Lys Phe Tyr Val Leu Pro Leu
               530                 535                 540

Xaa Gly Lys Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr
           545                 550                 555

Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn
       560                 565                 570

Ala Ala Gly His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly
   575                 580                 585

Ala Gly Pro Thr Ser Xaa Ser Ala Val Gly Val Leu Ala Pro His Ser
90                 595                 600                 605

Ala Leu Ala Val Leu Glu Asp Thr Val Asp Tyr Pro Ala Arg Ala His
               610                 615                 620

Thr Phe Asp Asp Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly
           625                 630                 635

Cys Ala Phe Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Met Lys
       640                 645                 650

Val Gly Lys Thr Arg Glu Ser
   655                 660
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<210> 93
<211> 122
<212> PRT
<213> Hepatitis E virus

<220>
<223> ORF3 HEV US-1

<400> 93

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Met Asn Asn Met Ser Phe Ala Ser Pro Met Gly Ser Pro Cys Ala Leu
  1               5                  10                  15

Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys Pro Arg
             20                  25                  30

His Arg Pro Val Ser Arg Leu Ala Val Ala Val Gly Gly Ala Ala Ala
         35                  40                  45

Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro Ser
     50                  55                  60

Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met Ser Phe
 65                  70                  75                  80
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His Asn Pro Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala Pro Ser
85 90 95

Val Pro Leu Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Pro Pro Val
100 105 110

Val Asp Leu Pro Gln Leu Gly Leu Arg Arg
115 120

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<210> 95
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<212> DNA
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<210> 96
<211> 20
<212> DNA
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<220>
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<210> 97
<211> 20
<212> DNA
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<220>
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tcatcctatc cagcgggtta tacataatga attagagcag tactgccggg cccgggctgg 180

tcgttgcttg gaggttgga 199

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<211> 25
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<220>
<223> JE orf1-s

<400> 99
gttctgcatc ggtgtttcct tagac 25

<210> 100
<211> 26
<212> DNA
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<400> 100
gaatcaggag atacgaggtt gtgtgg 26

<210> 101
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<212> DNA
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gaccgcacct attgttttga tggattttcc cgttgtgctt ttgctgcaga gaccggtgtg 180

gccctttact ctttgcatga cctttggcca gctgatgttg cagaggctat ggcccgccat 240

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accatcgggc ctgttcgggc taccacatgt gagctgtacg agctggttga agccatggta 240

gagaagggtc aggacggatc tgccgtccta gagctcgacc tttgcaatcg tgacgtctcg 300

cgcatcacat ttttccaaaa ggattgcaat aagtttacaa ctggtgagac tatcgcccat 360

ggcaaggttg gccagggcat atcggcctgg agcaagacct tctgtgctct gtttggcccg 420

tggttccgcg ccattgaaaa ggaaatattg gccctactcc cgcctaatat cttttatggc 480

gacgcctatg aggagtcagt gtttgctgcc gctgtgtccg gggcagggtc atgtatggta 540

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aatgttgcgc aggtctgtgt tgatgttgtg tcccgtgtct atggagttag ccccgggctg 1140

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<220>
<223> JE hevdf2/3 s1

<400> 103
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<210> 104
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<210> 105
<211> 130
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atgttgactc 130

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<223> hev216 a1

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<210> 108
<211> 564
<212> DNA
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gctgtcccgg ttcacatctg ggatcrgctc atgctctttg gtgccaccct gracgatcag 180

gcgttctgct gttcacggct tatgacttac ctccgtggta ttagttataa ggtcactgtc 240

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actgcggcct atctgaccat ctgccatcag cgttaccttc gcacccaggc gatttccaag 360

ggcatgcgcc ggttggaggt tgagcatgct cagaaattta tcacaagact ctacagctgg 420

ctatttgaga agtctggccg tgactacatc cccggccgcc agcttcaatt ttatgcacaa 480

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tcagtaccat gccgctgtag gacg 564

<210> 109
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<212> DNA
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<220>
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<400> 109
gtggagctag tacaccgacc gcag 24

<210> 110
<211> 678
<212> DNA

<213> Hepatitis E virus

<220>
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atgcgcagca agacaagggc atcaccattc cacatgatat agacctgggt gactcccgtg 180

tggttatcca ggattatgat aaccagcayg agcaagaccg acctactccg tcacctgccc 240

cctctcgccc cttctcagtt cttcgtgcca atgatgtttt gtggctttcc ctcactgccg 300

ctgagtatga ccagactacg tatgggtcgt ccaccaaccc tatgtatgtc tctgacacag 360

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aagttactct ggacggccgc ccccttacta ccattcagca gtattctaag acattttatg 480

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cttacaatta taatactacc gctagtgacc aaattttgat tgagaatgcg gccggccacc 600

gtgtcgctat ttccacctat accactagct taggtgccgg tcctacctcg atctctgcgg 660

tcggtgtact agctccac 678

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<220>
<223> hev3301s

<400> 111
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<210> 112
<211> 25
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<220>
<223> JE hev167-a1

<400> 112
ccaacacact atcggacaca gtgag 25

<210> 113
<211> 580

<212> DNA
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<223> us2-579

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gctgccaacc ctggtgcgat tacggttcac gaagctcagg gtgctacttt cacggagacc 180

acaattatag ccacggccga cgctaggggc ctcattcagt catcccgggc ccatgctata 240

gtcgcactca cccgccatac tgagaagtgt gttattttgg atgcccccgg cttgttgcgc 300

gaggtcggca tttcggatgt tattgtcaat aacttttttcc ttgccggtgg agaggtcggc 360

catcaccgcc cttctgtgat acctcgcggc aatcctgatc agaacctcgg gactctacag 420

gcctttccgc cgtcatgtca gatcagtgct taccatcagt tggctgagga actaggtcat 480

cgcccggccc ctgtcgccgc cgtcttgccc ccttgccctg agcttgagca gggcctgctc 540

tatatgccac aagaactcac tgtgtccgat agtgtgttgg 580

<210> 114
<211> 26
<212> DNA
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<220>
<223> HEV459 s1

<400> 114
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<210> 115
<211> 26
<212> DNA
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<220>
<223> HEV459 s3

<400> 115
ctctacagtt ggctatttga gaagtc 26

<210> 116
<211> 25
<212> DNA
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<220>
<223> JE1955a

<400> 116
ctataaagag ctgagcagaa ggcgg 25

<210> 117
<211> 734
<212> DNA
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<220>
<223> us2-733

<400> 117
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gtctttgatg aatcagtgcc atgccgttgc aggacgtttt tgaagaaggt cgcgggtaaa 180

ttctgctgtt ttatgcggtg gctggggcag gagtgtacct gcttcttgga gccagccgag 240

ggtttagttg gtgatcaagg tcatgacaac gaggcctatg aaggttctga ggtcgaccca 300

gctgagcctg cacatcttga tgtctcgggg acttatgccg tccatgggca ccagcttgag 360

gccctctata gggcacttaa tgtcccacat gatattgccg ctcgagcctc ccgactaacg 420

gctactgttg agctcgttgc tagtccggac cgcttagagt gccgcactgt acttggtaat 480

aagaccttcc ggacgacggt ggttgatggc gcccatcttg aagcgaatgg ccctgaggag 540

tatgttctgt catttgacgc ctctcgccag tctatggggg ccgggtcgca cagcctcact 600

tatgagctca cccctgccgg tctgcaggta aagatttcat ctaatggtct ggattgcact 660

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<400> 118
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<210> 119
<211> 22

<212> DNA
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<220>
<223> JE us2-579-a2

<400> 119
cagggttggc agccttagca gc 22

<210> 120
<211> 483
<212> DNA
<213> Hepatitis E virus

<220>
<223> us2-482

<400> 120
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acacagcggc ccgtgttact atcggccgcc gcgttgtgat tgatgaggct ccatctctcc 180

caccgcacct gctgctgtta cacatgcagc gggcctcctc ggtccatctc cttggtgatc 240

caaaccagat tcctgctatt gattttgagc atgccggcct ggtccccgcg atccgccccg 300

agcttgcgcc aacgagctgg tggcacgtta cacaccgttg cccggccgat gtgtgcgagc 360

tcatacgtgg ggcctacccc aaaattcaga ccacgagccg tgtgctacgg tccctgtttt 420

ggaacgaacc ggccatcggc caaaagttgg tttttacgca ggctgctaag gctgccaacc 480

ctg 483

<210> 121
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<223> us2-482-a2

<400> 123
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<210> 124
<211> 431
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<220>
<223> us2-430

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ggaacgcaat caccgccccg gcgatgagct ttacttgaca gagcccgccg cagcctggtt 180

tgaggctaat aagccggcgc agccggcgct tactataact gaggacacgg cccgtacggc 240

caacctggca ttagagattg atgccgccac agaggttggc cgtgcttgtg ccggctgcac 300

catcagcccc gggattgtgc actatcagtt taccgccggg gtcccgggct caggcaagtc 360

aaggtccata caacagggag atgtcgatgt ggtggttgtg cccacccggg agctccgtaa 420

cagctggcgt c 431

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<212> DNA
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<220>
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<400> 125
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<210> 126
<211> 25

<212> DNA
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<400> 126
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<210> 128
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<223> us2 330s1

<400> 130
cagctgatgt tgcagaggct atgg 24

<210> 131
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<223> us2 563a1

<400> 131
gcaggctgat ggaaacaagg atgg 24

<210> 132
<211> 407
<212> DNA
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<223> us2-406

<400> 132
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ctgaaccgtc acctatgcct tatgttccct accctcgttc aacggaggtg tatgtccggt 360

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<210> 135
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<223> us2-579 s2

<400> 135
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<223> us2-579wb

<400> 136

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<223> us2-430a1

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<210> 139
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<400> 139
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<210> 140
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<223> us2-430a2

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<210> 141
<211> 903
<212> DNA
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aggttcaccc agcggcattc gctgacaggc ggactatggc tacatcctga ggggctgctg 180

ggtatcttcc ccccattctc ccctgggcat atttgggagt ctgctaaccc cttttgcggt 240

gaggggactt tgtatacccg aacctggtca acctctggtt tttctagtga tttctccccc 300

cctgaggcgg ccgctcctgc ttcggctgcc gccccggggt tgccctaccc tactccacct 360

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<213> Hepatitis E virus

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<400> 142
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<210> 143
<211> 21
<212> DNA
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<210> 144
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Leu Val Phe Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val
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Ile His Asn Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys
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Leu Glu Val Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val
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Leu His Arg Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp
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Tyr Ser Ala Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala
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His Asp Leu Trp Pro Ala Asp Val Ala Glu Ala Met Ala Arg His Gly
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Pro Pro Gly Thr Tyr His Thr Thr Ser Tyr Leu Leu Ile His Asp Gly
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His Asp Val Ser Ile Leu Arg Ala Trp Ile Arg Thr Thr Lys Ile Val
                235                 240                 245

ggt gac cat cca ttg gtc ata gag cga gtg cgg gcc att ggg tgt cat   821
Gly Asp His Pro Leu Val Ile Glu Arg Val Arg Ala Ile Gly Cys His
            250                 255                 260
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ttt gtg ctg ctg ctc acc gca gcc cct gaa ccg tca cct atg cct tat   869
Phe Val Leu Leu Leu Thr Ala Ala Pro Glu Pro Ser Pro Met Pro Tyr
        265                 270                 275

gtt ccc tac cct cgt tca acg gag gtg tat gtc cgg tct ata ttt ggc   917
Val Pro Tyr Pro Arg Ser Thr Glu Val Tyr Val Arg Ser Ile Phe Gly
    280                 285                 290

cct ggc ggc tcc cca tcc ttg ttt cca tca gcc tgc tct act aaa tct   965
Pro Gly Gly Ser Pro Ser Leu Phe Pro Ser Ala Cys Ser Thr Lys Ser
295                 300                 305                 310

acc ttt cat gct gtc ccg gtt cac atc tgg gat crg ctc atg ctc ttt   1013
Thr Phe His Ala Val Pro Val His Ile Trp Asp Xaa Leu Met Leu Phe
                315                 320                 325

ggt gcc acc ctg rac gat cag gcg ttc tgc tgt tca cgg ctt atg act   1061
Gly Ala Thr Leu Xaa Asp Gln Ala Phe Cys Cys Ser Arg Leu Met Thr
            330                 335                 340

tac ctc cgt ggt att agt tat aag gtc act gtc ggt gcg ctt gtc gct   1109
Tyr Leu Arg Gly Ile Ser Tyr Lys Val Thr Val Gly Ala Leu Val Ala
        345                 350                 355

aat gag ggg tgg aac gcc tct gag gat gct ctt act gca gtg atc act   1157
Asn Glu Gly Trp Asn Ala Ser Glu Asp Ala Leu Thr Ala Val Ile Thr
    360                 365                 370

gcg gcc tat ctg acc atc tgc cat cag cgt tac ctt cgc acc cag gcg   1205
Ala Ala Tyr Leu Thr Ile Cys His Gln Arg Tyr Leu Arg Thr Gln Ala
375                 380                 385                 390

att tcc aag ggc atg cgc cgg ttg gag gtt gag cat gct cag aaa ttt   1253
Ile Ser Lys Gly Met Arg Arg Leu Glu Val Glu His Ala Gln Lys Phe
                395                 400                 405

atc aca aga ctc tac agc tgg cta ttt gag aag tct ggc cgt gac tac   1301
Ile Thr Arg Leu Tyr Ser Trp Leu Phe Glu Lys Ser Gly Arg Asp Tyr
            410                 415                 420

atc ccc ggc cgc cag ctt caa ttt tat gca caa tgc cga cgg tgg ctt   1349
Ile Pro Gly Arg Gln Leu Gln Phe Tyr Ala Gln Cys Arg Arg Trp Leu
        425                 430                 435

tct gca ggc ttc cac cta rac ccc agg rtg ctt gtc ttt gat gaa tca   1397
Ser Ala Gly Phe His Leu Xaa Pro Arg Xaa Leu Val Phe Asp Glu Ser
    440                 445                 450

gtg cca tgc cgt tgc agg acg ttt ttg aag aag gtc gcg ggt aaa ttc   1445
Val Pro Cys Arg Cys Arg Thr Phe Leu Lys Lys Val Ala Gly Lys Phe
455                 460                 465                 470

tgc tgt ttt atg cgg tgg ctg ggg cag gag tgt acc tgc ttc ttg gag   1493
Cys Cys Phe Met Arg Trp Leu Gly Gln Glu Cys Thr Cys Phe Leu Glu
                475                 480                 485
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cca gcc gag ggt tta gtt ggt gat caa ggt cat gac aac gag gcc tat   1541
Pro Ala Glu Gly Leu Val Gly Asp Gln Gly His Asp Asn Glu Ala Tyr
            490                 495                 500

gaa ggt tct gag gtc gac cca gct gag cct gca cat ctt gat gtc tcg   1589
Glu Gly Ser Glu Val Asp Pro Ala Glu Pro Ala His Leu Asp Val Ser
        505                 510                 515

ggg act tat gcc gtc cat ggg cac cag ctt gag gcc ctc tat agg gca   1637
Gly Thr Tyr Ala Val His Gly His Gln Leu Glu Ala Leu Tyr Arg Ala
    520                 525                 530

ctt aat gtc cca cat gat att gcc gct cga gcc tcc cga cta acg gct   1685
Leu Asn Val Pro His Asp Ile Ala Ala Arg Ala Ser Arg Leu Thr Ala
535                 540                 545                 550

act gtt gag ctc gtt gct agt ccg gac cgc tta gag tgc cgc act gta   1733
Thr Val Glu Leu Val Ala Ser Pro Asp Arg Leu Glu Cys Arg Thr Val
                555                 560                 565

ctt ggt aat aag acc ttc cgg acg acg gtg gtt gat ggc gcc cat ctt   1781
Leu Gly Asn Lys Thr Phe Arg Thr Thr Val Val Asp Gly Ala His Leu
            570                 575                 580

gaa gcg aat ggc cct gag gag tat gtt ctg tca ttt gac gcc tct cgc   1829
Glu Ala Asn Gly Pro Glu Glu Tyr Val Leu Ser Phe Asp Ala Ser Arg
        585                 590                 595

cag tct atg ggg gcc ggg tcg cac agc ctc act tat gag ctc acc cct   1877
Gln Ser Met Gly Ala Gly Ser His Ser Leu Thr Tyr Glu Leu Thr Pro
    600                 605                 610

gcc ggt ctg cag gta aag att tca tct aat ggt ctg gat tgc act gcc   1925
Ala Gly Leu Gln Val Lys Ile Ser Ser Asn Gly Leu Asp Cys Thr Ala
615                 620                 625                 630

aca ttc ccc ccy ggt ggc gcc cct agc gcc gcg ccg ggg gag gtg gcs   1973
Thr Phe Pro Xaa Gly Gly Ala Pro Ser Ala Ala Pro Gly Glu Val Xaa
                635                 640                 645

gcc ttc tgc agt gct ctt tat aga tac aat agg ttc acc cag cgg cat   2021
Ala Phe Cys Ser Ala Leu Tyr Arg Tyr Asn Arg Phe Thr Gln Arg His
            650                 655                 660

tcg ctg aca ggc gga cta tgg cta cat cct gag ggg ctg ctg ggt atc   2069
Ser Leu Thr Gly Gly Leu Trp Leu His Pro Glu Gly Leu Leu Gly Ile
        665                 670                 675

ttc ccc cca ttc tcc cct ggg cat att tgg gag tct gct aac ccc ttt   2117
Phe Pro Pro Phe Ser Pro Gly His Ile Trp Glu Ser Ala Asn Pro Phe
    680                 685                 690

tgc ggt gag ggg act ttg tat acc cga acc tgg tca acc tct ggt ttt   2165
Cys Gly Glu Gly Thr Leu Tyr Thr Arg Thr Trp Ser Thr Ser Gly Phe
695                 700                 705                 710

tct agt gat ttc tcc ccc cct gag gcg gcc gct cct gct tcg gct gcc   2213
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Ser Ser Asp Phe Ser Pro Pro Glu Ala Ala Ala Pro Ala Ser Ala Ala
                715                 720                 725

gcc ccg ggg ttg ccc tac cct act cca cct gtt agt gat atc tgg gtg   2261
Ala Pro Gly Leu Pro Tyr Pro Thr Pro Pro Val Ser Asp Ile Trp Val
            730                 735                 740

tta cca ccg ccc tca gag gaa tct cat gtt gat gcg gca tct gta ccc   2309
Leu Pro Pro Pro Ser Glu Glu Ser His Val Asp Ala Ala Ser Val Pro
        745                 750                 755

tct gtt cct gag cct gct gga ttg acc agc cct att gtg ctt acc ccc   2357
Ser Val Pro Glu Pro Ala Gly Leu Thr Ser Pro Ile Val Leu Thr Pro
    760                 765                 770

ccc ccc ccc cct cct ccc gtg cgt aag ccg gca aca tcc ccg cct ccc   2405
Pro Pro Pro Pro Pro Pro Val Arg Lys Pro Ala Thr Ser Pro Pro Pro
775                 780                 785                 790

cgc act cgc cgt ctc ctt tac acc tac ccc gac ggc gcc aag gtg tat   2453
Arg Thr Arg Arg Leu Leu Tyr Thr Tyr Pro Asp Gly Ala Lys Val Tyr
                795                 800                 805

gcg ggg tca ttg tkt gag tca gac tgt gat tgg tta gtc aat gcc tca   2501
Ala Gly Ser Leu Xaa Glu Ser Asp Cys Asp Trp Leu Val Asn Ala Ser
            810                 815                 820

aac cct ggc cat cgc ccc ggg ggt ggc ctc tgc cat gct ttt tat caa   2549
Asn Pro Gly His Arg Pro Gly Gly Gly Leu Cys His Ala Phe Tyr Gln
        825                 830                 835

cgt ttc cca gaa gcg ttc tac tcg act gaa ttc atc atg cgc gag ggc   2597
Arg Phe Pro Glu Ala Phe Tyr Ser Thr Glu Phe Ile Met Arg Glu Gly
    840                 845                 850

ctt gca gca tac act tta acc ccg cgc cct att atc cat gca gtg gct   2645
Leu Ala Ala Tyr Thr Leu Thr Pro Arg Pro Ile Ile His Ala Val Ala
855                 860                 865                 870

ccc gac tat agg gtt gag caa aac ccg aag agg ctt gag gca gcg tac   2693
Pro Asp Tyr Arg Val Glu Gln Asn Pro Lys Arg Leu Glu Ala Ala Tyr
                875                 880                 885

cgg gaa act tgc tcc cgt cgt ggc acc gct gcc tac ccg ctt ttg ggc   2741
Arg Glu Thr Cys Ser Arg Arg Gly Thr Ala Ala Tyr Pro Leu Leu Gly
            890                 895                 900

tcg ggt ata tac cag gtc cct gtt agc ctc agt ttt gat gcc tgg gaa   2789
Ser Gly Ile Tyr Gln Val Pro Val Ser Leu Ser Phe Asp Ala Trp Glu
        905                 910                 915

cgc aat cac cgc ccc ggc gat gag ctt tac ttg aca gag ccc gcc gca   2837
Arg Asn His Arg Pro Gly Asp Glu Leu Tyr Leu Thr Glu Pro Ala Ala
    920                 925                 930

gcc tgg ttt gag gct aat aag ccg gcg cag ccg gcg ctt act ata act   2885
Ala Trp Phe Glu Ala Asn Lys Pro Ala Gln Pro Ala Leu Thr Ile Thr
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935                 940                 945                 950

gag gac acg gcc cgt acg gcc aac ctg gca tta gag att gat gcc gcc   2933
Glu Asp Thr Ala Arg Thr Ala Asn Leu Ala Leu Glu Ile Asp Ala Ala
                955                 960                 965

aca gag gtt ggc cgt gct tgt gcc ggc tgc acc atc agc ccc ggg att   2981
Thr Glu Val Gly Arg Ala Cys Ala Gly Cys Thr Ile Ser Pro Gly Ile
            970                 975                 980

gtg cac tat cag ttt acc gcc ggg gtc ccg ggc tca ggc aag tca agg   3029
Val His Tyr Gln Phe Thr Ala Gly Val Pro Gly Ser Gly Lys Ser Arg
        985                 990                 995

tcc ata caa cag gga gat gtc gat gtg gtg gtt gtg ccc acc cgg gag   3077
Ser Ile Gln Gln Gly Asp Val Asp Val Val Val Val Pro Thr Arg Glu
   1000                1005                1010

ctc cgt aac agc tgg cgt cgc cgg ggt ttt gcg gcc ttc aca cct cac   3125
Leu Arg Asn Ser Trp Arg Arg Arg Gly Phe Ala Ala Phe Thr Pro His
1015                1020                1025                1030

aca gcg gcc cgt gtt act atc ggc cgc cgc gtt gtg att gat gag gct   3173
Thr Ala Ala Arg Val Thr Ile Gly Arg Arg Val Val Ile Asp Glu Ala
                1035                1040                1045

cca tct ctc cca ccg cac ctg ctg ctg tta cac atg cag cgg gcc tcc   3221
Pro Ser Leu Pro Pro His Leu Leu Leu Leu His Met Gln Arg Ala Ser
            1050                1055                1060

tcg gtc cat ctc ctt ggt gat cca aac cag att cct gct att gat ttt   3269
Ser Val His Leu Leu Gly Asp Pro Asn Gln Ile Pro Ala Ile Asp Phe
        1065                1070                1075

gag cat gcc ggc ctg gtc ccc gcg atc cgc ccc gag ctt gcg cca acg   3317
Glu His Ala Gly Leu Val Pro Ala Ile Arg Pro Glu Leu Ala Pro Thr
   1080                1085                1090

agc tgg tgg cac gtt aca cac cgt tgc ccg gcc gat gtg tgc gag ctc   3365
Ser Trp Trp His Val Thr His Arg Cys Pro Ala Asp Val Cys Glu Leu
1095                1100                1105                1110

ata cgt ggg gcc tac ccc aaa att cag acc acg agc cgt gtg cta cgg   3413
Ile Arg Gly Ala Tyr Pro Lys Ile Gln Thr Thr Ser Arg Val Leu Arg
                1115                1120                1125

tcc ctg ttt tgg aac gaa ccg gcc atc ggc caa aag ttg gtt ttt acg   3461
Ser Leu Phe Trp Asn Glu Pro Ala Ile Gly Gln Lys Leu Val Phe Thr
            1130                1135                1140

cag gct gct aag gct gcc aac cct ggt gcg att acg gtt cac gaa gct   3509
Gln Ala Ala Lys Ala Ala Asn Pro Gly Ala Ile Thr Val His Glu Ala
        1145                1150                1155

cag ggt gct act ttc acg gag acc aca att ata gcc acg gcc gac gct   3557
Gln Gly Ala Thr Phe Thr Glu Thr Thr Ile Ile Ala Thr Ala Asp Ala
   1160                1165                1170
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agg ggc ctc att cag tca tcc cgg gcc cat gct ata gtc gca ctc acc   3605
Arg Gly Leu Ile Gln Ser Ser Arg Ala His Ala Ile Val Ala Leu Thr
1175                1180                1185                1190

cgc cat act gag aag tgt gtt att ttg gat gcc ccc ggc ttg ttg cgc   3653
Arg His Thr Glu Lys Cys Val Ile Leu Asp Ala Pro Gly Leu Leu Arg
                1195                1200                1205

gag gtc ggc att tcg gat gtt att gtc aat aac ttt ttc ctt gcc ggt   3701
Glu Val Gly Ile Ser Asp Val Ile Val Asn Asn Phe Phe Leu Ala Gly
            1210                1215                1220

gga gag gtc ggc cat cac cgc cct tct gtg ata cct cgc ggc aat cct   3749
Gly Glu Val Gly His His Arg Pro Ser Val Ile Pro Arg Gly Asn Pro
        1225                1230                1235

gat cag aac ctc ggg act cta cag gcc ttt ccg ccg tca tgt cag atc   3797
Asp Gln Asn Leu Gly Thr Leu Gln Ala Phe Pro Pro Ser Cys Gln Ile
    1240                1245                1250

agt gct tac cat cag ttg gct gag gaa cta ggt cat cgc ccg gcc cct   3845
Ser Ala Tyr His Gln Leu Ala Glu Glu Leu Gly His Arg Pro Ala Pro
1255                1260                1265                1270

gtc gcc gcc gtc ttg ccc cct tgc cct gag ctt gag cag ggc ctg ctc   3893
Val Ala Ala Val Leu Pro Pro Cys Pro Glu Leu Glu Gln Gly Leu Leu
                1275                1280                1285

tat atg cca caa gaa ctt act gtg tcc gat agc gtg ctg gtt ttt gag   3941
Tyr Met Pro Gln Glu Leu Thr Val Ser Asp Ser Val Leu Val Phe Glu
            1290                1295                1300

ctt acg gat ata gtc cac tgc cgt atg gcc gcc cca agc cag cga aag   3989
Leu Thr Asp Ile Val His Cys Arg Met Ala Ala Pro Ser Gln Arg Lys
        1305                1310                1315

gct gtt ctc tca acg ctt gtg ggg agg tac ggc cgt agg act aaa tta   4037
Ala Val Leu Ser Thr Leu Val Gly Arg Tyr Gly Arg Arg Thr Lys Leu
    1320                1325                1330

tat gag gcg gcg cat tca gat gtc cgt gag tcc cta gcg agg ttt atc   4085
Tyr Glu Ala Ala His Ser Asp Val Arg Glu Ser Leu Ala Arg Phe Ile
1335                1340                1345                1350

ccc acc atc ggg cct gtt cgg gct acc aca tgt gag ctg tac gag ctg   4133
Pro Thr Ile Gly Pro Val Arg Ala Thr Thr Cys Glu Leu Tyr Glu Leu
                1355                1360                1365

gtt gaa gcc atg gta gag aag ggt cag gac gga tct gcc gtc cta gag   4181
Val Glu Ala Met Val Glu Lys Gly Gln Asp Gly Ser Ala Val Leu Glu
            1370                1375                1380

ctc gac ctt tgc aat cgt gac gtc tcg cgc atc aca ttt ttc caa aag   4229
Leu Asp Leu Cys Asn Arg Asp Val Ser Arg Ile Thr Phe Phe Gln Lys
        1385                1390                1395
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gat tgc aat aag ttt aca act ggt gag act atc gcc cat ggc aag gtt   4277
Asp Cys Asn Lys Phe Thr Thr Gly Glu Thr Ile Ala His Gly Lys Val
   1400                1405                1410

ggc cag ggc ata tcg gcc tgg agc aag acc ttc tgt gct ctg ttt ggc   4325
Gly Gln Gly Ile Ser Ala Trp Ser Lys Thr Phe Cys Ala Leu Phe Gly
1415                1420                1425                1430

ccg tgg ttc cgc gcc att gaa aag gaa ata ttg gcc cta ctc ccg cct   4373
Pro Trp Phe Arg Ala Ile Glu Lys Glu Ile Leu Ala Leu Leu Pro Pro
               1435                1440                1445

aat atc ttt tat ggc gac gcc tat gag gag tca gtg ttt gct gcc gct   4421
Asn Ile Phe Tyr Gly Asp Ala Tyr Glu Glu Ser Val Phe Ala Ala Ala
           1450                1455                1460

gtg tcc ggg gca ggg tca tgt atg gta ttt gaa aat gac ttc tca gag   4469
Val Ser Gly Ala Gly Ser Cys Met Val Phe Glu Asn Asp Phe Ser Glu
       1465                1470                1475

ttt gac agt acc cag aat aat ttc tct ctc ggc ctt gag tgt gtg gtt   4517
Phe Asp Ser Thr Gln Asn Asn Phe Ser Leu Gly Leu Glu Cys Val Val
   1480                1485                1490

atg gag gag tgc ggc atg ccc caa tgg tta att agg ttg tac cat ctg   4565
Met Glu Glu Cys Gly Met Pro Gln Trp Leu Ile Arg Leu Tyr His Leu
1495                1500                1505                1510

gtc cgg tca gcc tgg att ttg cag gcg ccg aag gag tct ctt aag ggg   4613
Val Arg Ser Ala Trp Ile Leu Gln Ala Pro Lys Glu Ser Leu Lys Gly
               1515                1520                1525

ttt tgg aag aag cac tct ggt gag cct ggt acc ctt ctc tgg aac act   4661
Phe Trp Lys Lys His Ser Gly Glu Pro Gly Thr Leu Leu Trp Asn Thr
           1530                1535                1540

gtc tgg aac atg gcg att ata gca cat tgc tay gag ttc cgt gac ttt   4709
Val Trp Asn Met Ala Ile Ile Ala His Cys Xaa Glu Phe Arg Asp Phe
       1545                1550                1555

cgt gtt gcc gcc ttc aag ggt gat gat tca gtg gtc ctc tgt agt gac   4757
Arg Val Ala Ala Phe Lys Gly Asp Asp Ser Val Val Leu Cys Ser Asp
   1560                1565                1570

tac cga cag rgc cgt aac gcg gct gcc tta att gca ggc tgt ggg ctc   4805
Tyr Arg Gln Xaa Arg Asn Ala Ala Ala Leu Ile Ala Gly Cys Gly Leu
1575                1580                1585                1590

aaa ttg aag gtt gat tac cgc cct atc ggg cta tat gct gga gtg gtg   4853
Lys Leu Lys Val Asp Tyr Arg Pro Ile Gly Leu Tyr Ala Gly Val Val
               1595                1600                1605

gtg gcc ccc ggt ttg ggg aca ctg ccc gat gtg gtg cgt ttt gcc ggt   4901
Val Ala Pro Gly Leu Gly Thr Leu Pro Asp Val Val Arg Phe Ala Gly
           1610                1615                1620

cgg tta tct gag aag aat tgg ggc cct ggc ccg gag cgt gct gag cag   4949
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Arg Leu Ser Glu Lys Asn Trp Gly Pro Gly Pro Glu Arg Ala Glu Gln
       1625                1630                1635

ctg cgt ctt gct gtt tgt gat ttc ctt cga ggg ttg acg aat gtt gcg   4997
Leu Arg Leu Ala Val Cys Asp Phe Leu Arg Gly Leu Thr Asn Val Ala
   1640                1645                1650

cag gtc tgt gtt gat gtt gtg tcc cgt gtc tat gga gtt agc ccc ggg   5045
Gln Val Cys Val Asp Val Val Ser Arg Val Tyr Gly Val Ser Pro Gly
1655                1660                1665                1670

ctg gta cat aac ctt att ggc atg ctg cag acc att gct gat ggc aag   5093
Leu Val His Asn Leu Ile Gly Met Leu Gln Thr Ile Ala Asp Gly Lys
               1675                1680                1685

gcc cac ttt aca gar aat att aaa cct gtg ctt gac ctt aca aat tcc   5141
Ala His Phe Thr Xaa Asn Ile Lys Pro Val Leu Asp Leu Thr Asn Ser
           1690                1695                1700

atc ata caa cgg gtg gaa tga ataacatgtc ttttgcatcg cccatgggat cacc 5196
Ile Ile Gln Arg Val Glu
       1705

atg cgc cct agg gct gtt ctg ttg ttg ctc ttc gtg ctt ttg cct atg   5244
Met Arg Pro Arg Ala Val Leu Leu Leu Leu Phe Val Leu Leu Pro Met
1710                1715                1720                1725

ctg ccc gcg cca ccg gcc ggc cag ccg tct ggc cgc cgt cgt ggg cgg   5292
Leu Pro Ala Pro Pro Ala Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg
               1730                1735                1740

cgc agc ggc ggt gcc ggc ggt ggt ttc tgg ggt gac agg gtt gat tct   5340
Arg Ser Gly Gly Ala Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser
           1745                1750                1755

cag ccc ttc gcc ctc ccc tat att cat cca acc aac ccc ttc gcc gcc   5388
Gln Pro Phe Ala Leu Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Ala
       1760                1765                1770

gat gtc gtt tca caa ccc ggg gct gga act cgc cct cga cag ccg ccc   5436
Asp Val Val Ser Gln Pro Gly Ala Gly Thr Arg Pro Arg Gln Pro Pro
   1775                1780                1785

cgc ccc ctt ggy tcc gct tgg cgt gac cag tcc cag cgc ccc tcc gct   5484
Arg Pro Leu Xaa Ser Ala Trp Arg Asp Gln Ser Gln Arg Pro Ser Ala
1790                1795                1800                1805

gcc ccc cgt cgt cga tct gcc cca gct ggg gct gcg ccg ctg act gcc   5532
Ala Pro Arg Arg Arg Ser Ala Pro Ala Gly Ala Ala Pro Leu Thr Ala
               1810                1815                1820

gtg tca ccg gct cct gac aca gcc cct gta cct gat gtt gac tca cgt   5580
Val Ser Pro Ala Pro Asp Thr Ala Pro Val Pro Asp Val Asp Ser Arg
           1825                1830                1835

ggt gct att ctg cgc cgg cag tac aat ttg tcc acg tcc ccg ctc acg   5628
Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr
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       1840                1845                1850

tca tct gtc gct tcg ggt act aat ttg gtc ctc tat gct gcc ccg ctg   5676
Ser Ser Val Ala Ser Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu
   1855                1860                1865

aat ccc ctc ttg cct ctc cag gat ggt acc aac act cat att atg gct   5724
Asn Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala
1870                1875                1880                1885

act gag gca tcc aat tat gcc cag tat cgg gtt gtt cga gct aca atc   5772
Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Val Arg Ala Thr Ile
               1890                1895                1900

cgt tat cgc ccg ctg gtg ccg aat gcc gtt ggt ggc tat gcc att tcc   5820
Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser
           1905                1910                1915

att tct ttc tgg ccc caa act aca act acc cct act tct gtc gat atg   5868
Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met
       1920                1925                1930

aat tct att act tcc acy gat gtt agg att ttg gtt cag ccc ggt att   5916
Asn Ser Ile Thr Ser Xaa Asp Val Arg Ile Leu Val Gln Pro Gly Ile
   1935                1940                1945

gcc tcc gag cta gtc atc ccc agt gag cgc ctt cat tac cgt aat caa   5964
Ala Ser Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln
1950                1955                1960                1965

ggc tgg cgc tct gtt gag acc acg ggt gtg gct gag gag gag gct act   6012
Gly Trp Arg Ser Val Glu Thr Thr Gly Val Ala Glu Glu Glu Ala Thr
               1970                1975                1980

tcc ggt ctg gta atg ctt tgc att cat ggc tct cct gtt aat tcc tac   6060
Ser Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr
           1985                1990                1995

act aat aca cct tac act ggt gcg ctg ggg ctt ctt gat ttt gca cta   6108
Thr Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu
       2000                2005                2010

gag ctt gaa ttt agg aat ttg aca ccc ggg aac acc aac acc cgt gtt   6156
Glu Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val
   2015                2020                2025

tcc cgg tat acc agc aca gcc cgc cac cgg ctg cgc cgt ggt gct gat   6204
Ser Arg Tyr Thr Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp
2030                2035                2040                2045

ggg act gct gag ctt act acc aca gca gcc aca cgt ttc atg aag gac   6252
Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp
               2050                2055                2060

ctg cac ttc gct ggc acg aat ggc gtt ggt gag gtg ggt cgt ggt atc   6300
Leu His Phe Ala Gly Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile
           2065                2070                2075
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gcc ctg aca ctg ttc aat ctc gct gat acg ctt ctc ggc ggt tta ccg   6348
Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro
       2080                2085                2090

aca gaa ttg att tcg tcg gct ggg ggc caa ctg ttt tac tcc cgc ccg   6396
Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
   2095                2100                2105

gtt gtc tca gcc aat ggc gag cca aca gta aag tta tat aca tct gtt   6444
Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
2110                2115                2120                2125

gag aat gcg cag caa gac aag ggc atc acc att cca cat gat ata gac   6492
Glu Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp
               2130                2135                2140

ctg ggt gac tcc cgt gtg gtt atc cag gat tat gat aac cag cay gag   6540
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln Xaa Glu
           2145                2150                2155

caa gac cga cct act ccg tca cct gcc ccc tct cgc ccc ttc tca gtt   6588
Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val
       2160                2165                2170

ctt cgt gcc aat gat gtt ttg tgg ctt tcc ctc act gcc gct gag tat   6636
Leu Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr
   2175                2180                2185

gac cag act acg tat ggg tcg tcc acc aac cct atg tat gtc tct gac   6684
Asp Gln Thr Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp
2190                2195                2200                2205

aca gtt acg ctt gtt aat gtg gct act ggt gct cag gct gtt gcc cgc   6732
Thr Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg
               2210                2215                2220

tcc ctt gat tgg tct aaa gtt act ctg gac ggc cgc ccc ctt act acc   6780
Ser Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr
           2225                2230                2235

att cag cag tat tct aag aca ttt tat gtt ctc ccg ctc cgc ggg aag   6828
Ile Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys
       2240                2245                2250

ctg tcc ttt tgg gag gct ggc acg act aag gcc ggc tac cct tac aat   6876
Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn
   2255                2260                2265

tat aat act acc gct agt gac caa att ttg att gag aat gcg gcc ggc   6924
Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly
2270                2275                2280                2285

cac cgt gtc gct att tcc acc tat acc act agc tta ggt gcc ggt cct   6972
His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro
               2290                2295                2300
```

```
acc tcg atc tct gcg gtc ggc gta ctg gct cca cac tct gcc ctt gcc   7020
Thr Ser Ile Ser Ala Val Gly Val Leu Ala Pro His Ser Ala Leu Ala
           2305                2310                2315

gtt ctt gag gat act att gat tac ccc gcc cgt gcc cat act ttt gat   7068
Val Leu Glu Asp Thr Ile Asp Tyr Pro Ala Arg Ala His Thr Phe Asp
       2320                2325                2330

gat ttt tgc ccg gag tgc cgt acc cta ggt ttg cag ggt tgt gca ttc   7116
Asp Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe
   2335                2340                2345

cag tct act att gct gag ctc cag cgt tta aaa atg aag gta ggt aaa   7164
Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys
2350                2355                2360                2365

acc cgg gag tct taa ttaattcctt ctgtgccccc ttcgtagttt ctttcgcttt   7219
Thr Arg Glu Ser
               2370

tatttcttat ttctgctttc cgcgctccct ggaaaaaaaa aaaaaaaaaa aaaaaaaa   7277


<210> 166
<211> 1708
<212> PRT
<213> Hepatitis E virus

<400> 166
Met Glu Ala His Gln Phe Ile Lys Ala Pro Gly Ile Thr Thr Ala Ile
  1               5                  10                  15

Glu Gln Ala Ala Leu Ala Ala Ala Asn Ser Ala Leu Ala Asn Ala Val
             20                  25                  30

Val Val Arg Pro Phe Leu Ser Arg Val Gln Thr Glu Ile Leu Ile Asn
         35                  40                  45

Leu Met Gln Pro Arg Gln Leu Val Phe Arg Pro Glu Val Leu Trp Asn
     50                  55                  60

His Pro Ile Gln Arg Val Ile His Asn Glu Leu Glu Gln Tyr Cys Arg
 65                  70                  75                  80

Ala Arg Ala Gly Arg Cys Leu Glu Val Gly Ala His Pro Arg Ser Ile
                 85                  90                  95

Asn Asp Asn Pro Asn Val Leu His Arg Cys Phe Leu Arg Pro Val Gly
            100                 105                 110

Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro Thr Arg Gly Pro Ala Ala
        115                 120                 125

Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu Pro Pro Val Asp Arg Thr
    130                 135                 140

Tyr Cys Phe Asp Gly Phe Ser Arg Cys Ala Phe Ala Ala Glu Thr Gly
```

```
145                 150                 155                 160

Val Ala Leu Tyr Ser Leu His Asp Leu Trp Pro Ala Asp Val Ala Glu
                165                 170                 175

Ala Met Ala Arg His Gly Met Thr Arg Leu Tyr Ala Ala Leu His Leu
            180                 185                 190

Pro Pro Glu Val Leu Leu Pro Pro Gly Thr Tyr His Thr Thr Ser Tyr
        195                 200                 205

Leu Leu Ile His Asp Gly Asn Arg Ala Val Val Thr Tyr Glu Gly Asp
    210                 215                 220

Thr Ser Ala Gly Tyr Asn His Asp Val Ser Ile Leu Arg Ala Trp Ile
225                 230                 235                 240

Arg Thr Thr Lys Ile Val Gly Asp His Pro Leu Val Ile Glu Arg Val
                245                 250                 255

Arg Ala Ile Gly Cys His Phe Val Leu Leu Leu Thr Ala Ala Pro Glu
            260                 265                 270

Pro Ser Pro Met Pro Tyr Val Pro Tyr Pro Arg Ser Thr Glu Val Tyr
        275                 280                 285

Val Arg Ser Ile Phe Gly Pro Gly Gly Ser Pro Ser Leu Phe Pro Ser
    290                 295                 300

Ala Cys Ser Thr Lys Ser Thr Phe His Ala Val Pro Val His Ile Trp
305                 310                 315                 320

Asp Xaa Leu Met Leu Phe Gly Ala Thr Leu Xaa Asp Gln Ala Phe Cys
                325                 330                 335

Cys Ser Arg Leu Met Thr Tyr Leu Arg Gly Ile Ser Tyr Lys Val Thr
            340                 345                 350

Val Gly Ala Leu Val Ala Asn Glu Gly Trp Asn Ala Ser Glu Asp Ala
        355                 360                 365

Leu Thr Ala Val Ile Thr Ala Ala Tyr Leu Thr Ile Cys His Gln Arg
    370                 375                 380

Tyr Leu Arg Thr Gln Ala Ile Ser Lys Gly Met Arg Arg Leu Glu Val
385                 390                 395                 400

Glu His Ala Gln Lys Phe Ile Thr Arg Leu Tyr Ser Trp Leu Phe Glu
                405                 410                 415

Lys Ser Gly Arg Asp Tyr Ile Pro Gly Arg Gln Leu Gln Phe Tyr Ala
            420                 425                 430

Gln Cys Arg Arg Trp Leu Ser Ala Gly Phe His Leu Xaa Pro Arg Xaa
        435                 440                 445

Leu Val Phe Asp Glu Ser Val Pro Cys Arg Cys Arg Thr Phe Leu Lys
```

```
    450                 455                 460

Lys Val Ala Gly Lys Phe Cys Cys Phe Met Arg Trp Leu Gly Gln Glu
465                 470                 475                 480

Cys Thr Cys Phe Leu Glu Pro Ala Glu Gly Leu Val Gly Asp Gln Gly
                485                 490                 495

His Asp Asn Glu Ala Tyr Glu Gly Ser Glu Val Asp Pro Ala Glu Pro
            500                 505                 510

Ala His Leu Asp Val Ser Gly Thr Tyr Ala Val His Gly His Gln Leu
        515                 520                 525

Glu Ala Leu Tyr Arg Ala Leu Asn Val Pro His Asp Ile Ala Ala Arg
    530                 535                 540

Ala Ser Arg Leu Thr Ala Thr Val Glu Leu Val Ala Ser Pro Asp Arg
545                 550                 555                 560

Leu Glu Cys Arg Thr Val Leu Gly Asn Lys Thr Phe Arg Thr Thr Val
                565                 570                 575

Val Asp Gly Ala His Leu Glu Ala Asn Gly Pro Glu Glu Tyr Val Leu
            580                 585                 590

Ser Phe Asp Ala Ser Arg Gln Ser Met Gly Ala Gly Ser His Ser Leu
        595                 600                 605

Thr Tyr Glu Leu Thr Pro Ala Gly Leu Gln Val Lys Ile Ser Ser Asn
    610                 615                 620

Gly Leu Asp Cys Thr Ala Thr Phe Pro Xaa Gly Gly Ala Pro Ser Ala
625                 630                 635                 640

Ala Pro Gly Glu Val Xaa Ala Phe Cys Ser Ala Leu Tyr Arg Tyr Asn
                645                 650                 655

Arg Phe Thr Gln Arg His Ser Leu Thr Gly Gly Leu Trp Leu His Pro
            660                 665                 670

Glu Gly Leu Leu Gly Ile Phe Pro Pro Phe Ser Pro Gly His Ile Trp
        675                 680                 685

Glu Ser Ala Asn Pro Phe Cys Gly Glu Gly Thr Leu Tyr Thr Arg Thr
    690                 695                 700

Trp Ser Thr Ser Gly Phe Ser Ser Asp Phe Ser Pro Pro Glu Ala Ala
705                 710                 715                 720

Ala Pro Ala Ser Ala Ala Ala Pro Gly Leu Pro Tyr Pro Thr Pro Pro
                725                 730                 735

Val Ser Asp Ile Trp Val Leu Pro Pro Pro Ser Glu Glu Ser His Val
            740                 745                 750

Asp Ala Ala Ser Val Pro Ser Val Pro Glu Pro Ala Gly Leu Thr Ser
```

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        755                 760                 765

Pro Ile Val Leu Thr Pro Pro Pro Pro Pro Pro Pro Val Arg Lys Pro
    770                 775                 780

Ala Thr Ser Pro Pro Pro Arg Thr Arg Arg Leu Leu Tyr Thr Tyr Pro
785                 790                 795                 800

Asp Gly Ala Lys Val Tyr Ala Gly Ser Leu Xaa Glu Ser Asp Cys Asp
                805                 810                 815

Trp Leu Val Asn Ala Ser Asn Pro Gly His Arg Pro Gly Gly Gly Leu
            820                 825                 830

Cys His Ala Phe Tyr Gln Arg Phe Pro Glu Ala Phe Tyr Ser Thr Glu
        835                 840                 845

Phe Ile Met Arg Glu Gly Leu Ala Ala Tyr Thr Leu Thr Pro Arg Pro
    850                 855                 860

Ile Ile His Ala Val Ala Pro Asp Tyr Arg Val Glu Gln Asn Pro Lys
865                 870                 875                 880

Arg Leu Glu Ala Ala Tyr Arg Glu Thr Cys Ser Arg Arg Gly Thr Ala
                885                 890                 895

Ala Tyr Pro Leu Leu Gly Ser Gly Ile Tyr Gln Val Pro Val Ser Leu
            900                 905                 910

Ser Phe Asp Ala Trp Glu Arg Asn His Arg Pro Gly Asp Glu Leu Tyr
        915                 920                 925

Leu Thr Glu Pro Ala Ala Ala Trp Phe Glu Ala Asn Lys Pro Ala Gln
    930                 935                 940

Pro Ala Leu Thr Ile Thr Glu Asp Thr Ala Arg Thr Ala Asn Leu Ala
945                 950                 955                 960

Leu Glu Ile Asp Ala Ala Thr Glu Val Gly Arg Ala Cys Ala Gly Cys
                965                 970                 975

Thr Ile Ser Pro Gly Ile Val His Tyr Gln Phe Thr Ala Gly Val Pro
            980                 985                 990

Gly Ser Gly Lys Ser Arg Ser Ile Gln Gln Gly Asp Val Asp Val Val
        995                1000                1005

Val Val Pro Thr Arg Glu Leu Arg Asn Ser Trp Arg Arg Arg Gly Phe
   1010                1015                1020

Ala Ala Phe Thr Pro His Thr Ala Ala Arg Val Thr Ile Gly Arg Arg
025                1030                1035                1040

Val Val Ile Asp Glu Ala Pro Ser Leu Pro Pro His Leu Leu Leu Leu
               1045                1050                1055

His Met Gln Arg Ala Ser Ser Val His Leu Leu Gly Asp Pro Asn Gln
```

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          1060                1065                1070

Ile Pro Ala Ile Asp Phe Glu His Ala Gly Leu Val Pro Ala Ile Arg
       1075                1080                1085

Pro Glu Leu Ala Pro Thr Ser Trp Trp His Val Thr His Arg Cys Pro
   1090                1095                1100

Ala Asp Val Cys Glu Leu Ile Arg Gly Ala Tyr Pro Lys Ile Gln Thr
105                1110                1115                1120

Thr Ser Arg Val Leu Arg Ser Leu Phe Trp Asn Glu Pro Ala Ile Gly
               1125                1130                1135

Gln Lys Leu Val Phe Thr Gln Ala Ala Lys Ala Ala Asn Pro Gly Ala
           1140                1145                1150

Ile Thr Val His Glu Ala Gln Gly Ala Thr Phe Thr Glu Thr Thr Ile
       1155                1160                1165

Ile Ala Thr Ala Asp Ala Arg Gly Leu Ile Gln Ser Ser Arg Ala His
   1170                1175                1180

Ala Ile Val Ala Leu Thr Arg His Thr Glu Lys Cys Val Ile Leu Asp
185                1190                1195                1200

Ala Pro Gly Leu Leu Arg Glu Val Gly Ile Ser Asp Val Ile Val Asn
               1205                1210                1215

Asn Phe Phe Leu Ala Gly Gly Glu Val Gly His His Arg Pro Ser Val
           1220                1225                1230

Ile Pro Arg Gly Asn Pro Asp Gln Asn Leu Gly Thr Leu Gln Ala Phe
       1235                1240                1245

Pro Pro Ser Cys Gln Ile Ser Ala Tyr His Gln Leu Ala Glu Glu Leu
   1250                1255                1260

Gly His Arg Pro Ala Pro Val Ala Ala Val Leu Pro Pro Cys Pro Glu
265                1270                1275                1280

Leu Glu Gln Gly Leu Leu Tyr Met Pro Gln Glu Leu Thr Val Ser Asp
               1285                1290                1295

Ser Val Leu Val Phe Glu Leu Thr Asp Ile Val His Cys Arg Met Ala
           1300                1305                1310

Ala Pro Ser Gln Arg Lys Ala Val Leu Ser Thr Leu Val Gly Arg Tyr
       1315                1320                1325

Gly Arg Arg Thr Lys Leu Tyr Glu Ala Ala His Ser Asp Val Arg Glu
   1330                1335                1340

Ser Leu Ala Arg Phe Ile Pro Thr Ile Gly Pro Val Arg Ala Thr Thr
345                1350                1355                1360

Cys Glu Leu Tyr Glu Leu Val Glu Ala Met Val Glu Lys Gly Gln Asp
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1365 1370 1375

Gly Ser Ala Val Leu Glu Leu Asp Leu Cys Asn Arg Asp Val Ser Arg
1380 1385 1390

Ile Thr Phe Phe Gln Lys Asp Cys Asn Lys Phe Thr Thr Gly Glu Thr
1395 1400 1405

Ile Ala His Gly Lys Val Gly Gln Gly Ile Ser Ala Trp Ser Lys Thr
1410 1415 1420

Phe Cys Ala Leu Phe Gly Pro Trp Phe Arg Ala Ile Glu Lys Glu Ile
425 1430 1435 1440

Leu Ala Leu Leu Pro Pro Asn Ile Phe Tyr Gly Asp Ala Tyr Glu Glu
1445 1450 1455

Ser Val Phe Ala Ala Ala Val Ser Gly Ala Gly Ser Cys Met Val Phe
1460 1465 1470

Glu Asn Asp Phe Ser Glu Phe Asp Ser Thr Gln Asn Asn Phe Ser Leu
1475 1480 1485

Gly Leu Glu Cys Val Val Met Glu Glu Cys Gly Met Pro Gln Trp Leu
1490 1495 1500

Ile Arg Leu Tyr His Leu Val Arg Ser Ala Trp Ile Leu Gln Ala Pro
505 1510 1515 1520

Lys Glu Ser Leu Lys Gly Phe Trp Lys Lys His Ser Gly Glu Pro Gly
1525 1530 1535

Thr Leu Leu Trp Asn Thr Val Trp Asn Met Ala Ile Ile Ala His Cys
1540 1545 1550

Xaa Glu Phe Arg Asp Phe Arg Val Ala Ala Phe Lys Gly Asp Asp Ser
1555 1560 1565

Val Val Leu Cys Ser Asp Tyr Arg Gln Xaa Arg Asn Ala Ala Ala Leu
1570 1575 1580

Ile Ala Gly Cys Gly Leu Lys Leu Lys Val Asp Tyr Arg Pro Ile Gly
585 1590 1595 1600

Leu Tyr Ala Gly Val Val Val Ala Pro Gly Leu Gly Thr Leu Pro Asp
1605 1610 1615

Val Val Arg Phe Ala Gly Arg Leu Ser Glu Lys Asn Trp Gly Pro Gly
1620 1625 1630

Pro Glu Arg Ala Glu Gln Leu Arg Leu Ala Val Cys Asp Phe Leu Arg
1635 1640 1645

Gly Leu Thr Asn Val Ala Gln Val Cys Val Asp Val Val Ser Arg Val
1650 1655 1660

Tyr Gly Val Ser Pro Gly Leu Val His Asn Leu Ile Gly Met Leu Gln

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665                 1670                 1675                 1680

Thr Ile Ala Asp Gly Lys Ala His Phe Thr Xaa Asn Ile Lys Pro Val
                1685                 1690                 1695

Leu Asp Leu Thr Asn Ser Ile Ile Gln Arg Val Glu
            1700                 1705


<210> 167
<211> 660
<212> PRT
<213> Hepatitis E virus

<400> 167
Met Arg Pro Arg Ala Val Leu Leu Leu Leu Phe Val Leu Leu Pro Met
  1               5                  10                  15

Leu Pro Ala Pro Pro Ala Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg
             20                  25                  30

Arg Ser Gly Gly Ala Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser
         35                  40                  45

Gln Pro Phe Ala Leu Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Ala
     50                  55                  60

Asp Val Val Ser Gln Pro Gly Ala Gly Thr Arg Pro Arg Gln Pro Pro
 65                  70                  75                  80

Arg Pro Leu Xaa Ser Ala Trp Arg Asp Gln Ser Gln Arg Pro Ser Ala
                 85                  90                  95

Ala Pro Arg Arg Arg Ser Ala Pro Ala Gly Ala Ala Pro Leu Thr Ala
            100                 105                 110

Val Ser Pro Ala Pro Asp Thr Ala Pro Val Pro Asp Val Asp Ser Arg
        115                 120                 125

Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr
    130                 135                 140

Ser Ser Val Ala Ser Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu
145                 150                 155                 160

Asn Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala
                165                 170                 175

Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Val Arg Ala Thr Ile
            180                 185                 190

Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser
        195                 200                 205

Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met
    210                 215                 220
```

```
Asn Ser Ile Thr Ser Xaa Asp Val Arg Ile Leu Val Gln Pro Gly Ile
225                 230                 235                 240

Ala Ser Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln
                245                 250                 255

Gly Trp Arg Ser Val Glu Thr Thr Gly Val Ala Glu Glu Glu Ala Thr
            260                 265                 270

Ser Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr
        275                 280                 285

Thr Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu
    290                 295                 300

Glu Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val
305                 310                 315                 320

Ser Arg Tyr Thr Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp
                325                 330                 335

Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp
            340                 345                 350

Leu His Phe Ala Gly Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile
        355                 360                 365

Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro
    370                 375                 380

Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
385                 390                 395                 400

Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
                405                 410                 415

Glu Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp
            420                 425                 430

Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln Xaa Glu
        435                 440                 445

Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val
    450                 455                 460

Leu Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr
465                 470                 475                 480

Asp Gln Thr Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp
                485                 490                 495

Thr Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg
            500                 505                 510

Ser Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr
        515                 520                 525
```

```
Ile Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys
    530                 535                 540

Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn
545                 550                 555                 560

Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly
                565                 570                 575

His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro
            580                 585                 590

Thr Ser Ile Ser Ala Val Gly Val Leu Ala Pro His Ser Ala Leu Ala
        595                 600                 605

Val Leu Glu Asp Thr Ile Asp Tyr Pro Ala Arg Ala His Thr Phe Asp
    610                 615                 620

Asp Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe
625                 630                 635                 640

Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys
                645                 650                 655

Thr Arg Glu Ser
            660
```

<210> 168
<211> 122
<212> PRT
<213> Hepatitis E virus

<220>
<223> us2 orf3

<400> 168

```
Met Asn Asn Met Ser Phe Ala Ser Pro Met Gly Ser Pro Cys Ala Leu
  1               5                  10                  15

Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys Pro Arg
             20                  25                  30

His Arg Pro Ala Ser Arg Leu Ala Ala Val Val Gly Gly Ala Ala Ala
         35                  40                  45

Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro Ser
     50                  55                  60

Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met Ser Phe
 65                  70                  75                  80

His Asn Pro Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala Pro Leu
                 85                  90                  95

Xaa Pro Leu Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Pro Pro Val
            100                 105                 110
```

Val Asp Leu Pro Gln Leu Gly Leu Arg Arg
115 120

<210> 169
<211> 33
<212> PRT
<213> Hepatitis E virus

<220>
<223> M 4-2

<400> 169
Ala Asn Gln Pro Gly His Leu Ala Pro Leu Gly Glu Ile Arg Pro Ser
1 5 10 15

Ala Pro Pro Leu Pro Pro Val Ala Asp Leu Pro Gln Pro Gly Leu Arg
20 25 30

Arg

<210> 170
<211> 48
<212> PRT
<213> Hepatitis E virus

<220>
<223> M 3-2e

<400> 170
Thr Phe Asp Tyr Pro Gly Arg Ala His Thr Phe Asp Asp Phe Cys Pro
1 5 10 15

Glu Cys Arg Ala Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Val
20 25 30

Ala Glu Leu Gln Arg Leu Lys Val Lys Val Gly Lys Thr Arg Glu Leu
35 40 45

<210> 171
<211> 33
<212> PRT
<213> Hepatitis E virus

<220>
<223> B 4-2

<400> 171
Ala Asn Pro Pro Asp His Ser Ala Pro Leu Gly Val Thr Arg Pro Ser
1 5 10 15

Ala Pro Pro Leu Pro His Val Val Asp Leu Pro Gln Leu Gly Pro Arg
20 25 30

Arg

<210> 172
<211> 48
<212> PRT
<213> Hepatitis E virus

<220>
<223> B 3-2e

<400> 172
Thr Leu Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro
1 5 10 15

Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Val
20 25 30

Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu Leu
35 40 45

<210> 173
<211> 33
<212> PRT
<213> Hepatitis E virus

<220>
<223> ORF3 (u4.2)

<400> 173
Asp Ser Arg Pro Ala Pro Ser Val Pro Leu Gly Val Thr Ser Pro Ser
1 5 10 15

Ala Pro Pro Leu Pro Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg
20 25 30

Arg

<210> 174
<211> 48
<212> PRT
<213> Hepatitis E virus

<220>
<223> ORF2 (u3.2e)

<400> 174
Thr Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro
1 5 10 15

Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Ile
20 25 30

Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu Ser
35 40 45

<210> 175
<211> 33
<212> PRT
<213> Hepatitis E virus

<220>
<223> US 4-2

<400> 175
Asp Ser Arg Pro Ala Pro Ser Val Pro Leu Gly Val Thr Ser Pro Ser
1 5 10 15

Ala Pro Pro Leu Pro Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg
20 25 30

Cys

<210> 176
<211> 48
<212> PRT
<213> Hepatitis E virus

<220>
<223> US 3-2e

<400> 176
Thr Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro
1 5 10 15

Glu Cys Arg Thr Leu Gly Val Gln Gly Cys Ala Phe Gln Ser Thr Ile
20 25 30

Ala Glu Val Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu Val
35 40 45

<210> 177
<211> 21

<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF1-s2

<400> 177
ctgccytkgc gaatgctgtg g 21

<210> 178
<211> 24
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF1-a2

<400> 178
ggcagwrtac carcgctgaa catc 24

<210> 179
<211> 294
<212> DNA
<213> Hepatitis E virus

<220>
<223> z12-orf1 (G.S.)

<400> 179
tggcattact actgccattg agcaagctgc tctggctgcg gccaattctg ccttggcgaa 60

tgctgtggtg gttcggccgt ttttatctcg tttacagact gagattctta ttaatttgat 120

gcaaccccga cagttggtct ttcgacctga ggtgttctgg aaccatccca tccaacgtgt 180

tatacataat gaattggagc agtactgccg ggcccgggcc ggtcgctgtc tggaaattgg 240

agcccatcca aggtcaatca atgataatcc taatgttctg catcggtgtt tcct 294

<210> 180
<211> 418
<212> DNA
<213> Hepatitis E virus

<220>
<223> z12-orf1.con

<400> 180
ctggcattac tactgctatt gagcaagctg ctctgggtgc ggccaattct gccttggcga 60

atgctgtggt ggttcggccg tttttatctc gtttacagac tgagattctt attaatttga 120

tgcaaccccg acagttggtc tttcgacctg aggtgttctg gaaccatccc atccaacgtg 180

ttatacataa tgaattggag cagtactgcc gggcccgggc cggtcgctgt ctggaaattg 240

gagcccatcc aaggtcaatc aatgataatc ctaatgttct gcatcggtgc tttttacgac 300

cggtcgggag ggacgttcag cgctggtact ccgcccccac ccgtggcccc gcggccaact 360

gccgccggtc tgcgctgcgt ggtctccccc ctgtcgaccg cacttactgc ctcgatgg 418

<210> 181
<211> 197
<212> DNA
<213> Hepatitis E virus

<220>
<223> z12-orf2.con

<400> 181
gacagaatta atttcgtcgg ctgggggtca actgttctac tcccgccctg tcgtctcagc 60

caatggcgag ccgactgtca agttatacac atctgttgag aatgcacagc aggataaggg 120

gatagctatt ccacatgaca tagatttggg cgactctcgt ttggtaatcc aggattatga 180

taaccaacac gaacaag 197

<210> 182
<211> 25
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF2/3-s1

<400> 182
gtatcggkyk gaatgaataa catgt 25

<210> 183
<211> 25
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF2/3-a1

<400> 183
aggggttggt tggatgaata taggg 25

<210> 184
<211> 234
<212> DNA
<213> Hepatitis E virus

<220>

<223> z12-orf23.con

<400> 184
gtatcggktt gaatgaataa catgttttgt gcatcgccca tgggatcacc atgcgcccta 60

gggttgttct gttgttgttc ctcgtgtttc tgcctatgct gcccgcgcca ccggccggcc 120

agycgactgg ccgccgtcgt gggcggcgca gcggcggtgc cggcggtggt ttctggggtg 180

acagggttga ttctcagccc ttcgccctcc cctatattca tccaaccaac ccct 234

<210> 185
<211> 890
<212> DNA
<213> Hepatitis E virus

<220>
<223> z12-3p.race

<400> 185
gtcgtctcgg ccaatggcga gccgactgtc aagttataca catctgttga gaatgcacag 60

caggataagg ggatagctat tccacatgac atagatttgg gcgactctcg tttggtaatc 120

caggattacg ataatcagca cgagcaggac cggcccaccc cttcgcccgc cccgtctcgt 180

cctttctcgg tcctccgcgc taatgatgct ttgtggcttt ctcttaccgc tgctgagtat 240

gaccagacta catatgggtc gtccaccaac ccgatgtatg tctcagacac tgttacattt 300

gtcaatgtgg ccacaggggc tcaggctgtc gcccgttctc ttgattggtc taaagttacc 360

ctggacggcc gccctcttac taccatccag cagtactcta agacatttta tgttctccca 420

cttcgcggga agttatcttt ttgggaggct ggcacaacta aagccggtta cccttataat 480

tataacacaa ctgctagtga ccagattctg attgaaaacg cggctggcca tcgtgtcgct 540

atatctactt atactactag cctgggcgcc ggccctgtgt cagtttctgc ggttggtgtg 600

ttagccccac actcgagcct tgctattctt gaagacactg ttgactatcc ggcccgtgct 660

cacacttttg atgacttctg tccggaatgc cgtgccctgg gtctgcaggg gtgtgctttt 720

caatctacta tcgctgagct ccagcgtctt aaaatgaagg taggcaaaac ccgggagttt 780

taattaattc ttcttgtgcc cccttcacgg ttctcgcttt atttctttct tctgcctccc 840

gcgctccctg gaaaaaaaaa aaaaaaaaaa gtactagtcg acgcgtggcc 890

<210> 186
<211> 919
<212> DNA
<213> Hepatitis E virus

<220>
<223> z12-3p.con

<400> 186
gacagaatta atttcgtcgg ctgggggtca actgttctac tcccgccctg tcgtctcagc 60

caatggcgag ccgactgtca agttatacac atctgttgag aatgcacagc aggataaggg 120

gatagctatt ccacatgaca tagatttggg cgactctcgt ttggtaatcc aggattacga 180

taatcagcac gagcaggacc ggcccacccc ttcgcccgcc ccgtctcgtc ctttctcggt 240

cctccgcgct aatgatgctt tgtggctttc tcttaccgct gctgagtatg accagactac 300

atatgggtcg tccaccaacc cgatgtatgt ctcagacact gttacatttg tcaatgtggc 360

cacaggggct caggctgtcg cccgttctct tgattggtct aaagttaccc tggacggccg 420

ccctcttact accatccagc agtactctaa gacattttat gttctcccac ttcgcgggaa 480

gttatctttt tgggaggctg gcacaactaa agccggttac ccttataatt ataacacaac 540

tgctagtgac cagattctga ttgaaaacgc ggctggccat cgtgtcgcta tatctactta 600

tactactagc ctgggcgccg gccctgtgtc agtttctgcg gttggtgtgt tagccccaca 660

ctcgagcctt gctattcttg aagacactgt tgactatccg gcccgtgctc acacttttga 720

tgacttctgt ccggaatgcc gtgccctggg tctgcagggg tgtgcttttc aatctactat 780

cgctgagctc cagcgtctta aaatgaaggt aggcaaaacc cgggagtttt aattaattct 840

tcttgtgccc ccttcacggt tctcgcttta tttctttctt ctgcctcccg cgctccctgg 900

aaaaaaaaaa aaaaaaaaa 919

<210> 187
<211> 138
<212> PRT
<213> Hepatitis E virus

<220>
<223> z12-orf1.pep

<400> 187
Gly Ile Thr Thr Ala Ile Glu Gln Ala Ala Leu Gly Ala Ala Asn Ser
1 5 10 15

Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Leu Gln
20 25 30

Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe Arg
35 40 45

Pro Glu Val Phe Trp Asn His Pro Ile Gln Arg Val Ile His Asn Glu
50 55 60

```
Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Ile Gly
 65                  70                  75                  80

Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg Cys
                85                  90                  95

Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro
            100                 105                 110

Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu
        115                 120                 125

Pro Pro Val Asp Arg Thr Tyr Cys Leu Asp
    130                 135


<210> 188
<211> 61
<212> PRT
<213> Hepatitis E virus

<220>
<223> z12-orf2-5'.pep

<400> 188
Met Arg Pro Arg Val Val Leu Leu Leu Phe Leu Val Phe Leu Pro Met
  1               5                  10                  15

Leu Pro Ala Pro Pro Ala Gly Gln Xaa Thr Gly Arg Arg Arg Gly Arg
             20                  25                  30

Arg Ser Gly Gly Ala Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser
         35                  40                  45

Gln Pro Phe Ala Leu Pro Tyr Ile His Pro Thr Asn Pro
     50                  55                  60


<210> 189
<211> 276
<212> PRT
<213> Hepatitis E virus

<220>
<223> z12-orf2-3'.pep

<400> 189
Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
  1               5                  10                  15

Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
             20                  25                  30

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35                  40                  45
```

```
Leu Gly Asp Ser Arg Leu Val Ile Gln Asp Tyr Asp Asn Gln His Glu
     50                  55                  60

Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val
 65                  70                  75                  80

Leu Arg Ala Asn Asp Ala Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr
                 85                  90                  95

Asp Gln Thr Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp
            100                 105                 110

Thr Val Thr Phe Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg
        115                 120                 125

Ser Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr
    130                 135                 140

Ile Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys
145                 150                 155                 160

Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn
                165                 170                 175

Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly
            180                 185                 190

His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro
        195                 200                 205

Val Ser Val Ser Ala Val Gly Val Leu Ala Pro His Ser Ser Leu Ala
    210                 215                 220

Ile Leu Glu Asp Thr Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp
225                 230                 235                 240

Asp Phe Cys Pro Glu Cys Arg Ala Leu Gly Leu Gln Gly Cys Ala Phe
                245                 250                 255

Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys
            260                 265                 270

Thr Arg Glu Phe
        275


<210> 190
<211> 74
<212> PRT
<213> Hepatitis E virus

<220>
<223> z12-orf3.pep

<400> 190
Met Asn Asn Met Phe Cys Ala Ser Pro Met Gly Ser Pro Cys Ala Leu
  1               5                  10                  15
```

Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys Pro Arg
20 25 30

His Arg Pro Ala Ser Arg Leu Ala Ala Val Val Gly Gly Ala Ala Ala
35 40 45

Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro Ser
50 55 60

Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro
65 70

<210> 191
<211> 408
<212> DNA
<213> Hepatitis E virus

<220>
<223> pJOorf3-29.seq

<400> 191
gaattcatga ataacatgtc ttttgcatcg cccatgggat caccatgcgc cctagggctg 60

ttctgttgtt gctcttcgtg cttttgccta tgctgcccgc gccaccggcc agccagccgt 120

ctggccgccg tcgtgggcgg cgcagcggcg gtgccggcgg tggtttctgg ggtgacaggg 180

ttgattctca gcccttcgcc ctcccctata ttcatccaac caacccccttc gccgccgatg 240

tcgtttcaca acccggggct ggaactcgcc ctcgacagcc gccccgcccc cttggctccg 300

cttggcgtga ccagtcccag cgcccctccg ctgccccccg tcgtcgatct gccccagctt 360

ggtctgcgcc gcgactacaa ggacgacgat gacaagtaat aaggatcc 408

<210> 192
<211> 1026
<212> DNA
<213> Hepatitis E virus

<220>
<223> cksorf2m-2.seq

<400> 192
gaattcatgg gtgctgatgg gactgctgag cttactacca cagcagccac acgtttcatg 60

aaggacctgc acttcgctgg cacgaatggc gttggtgagg tgggtcgtgg tatcgccctg 120

acactgttca atctcgctga tacgcttctc ggcggtttac cgacagaatt gatttcgtcg 180

gctgggggcc aactgtttta ctcccgcccg gttgtctcag ccaatggcga gccaacagta 240

aagttatata catctgttga gaatgcgcag caagacaagg gcatcaccat tccacatgat 300

atagacctgg gtgactcccg tgtggttatc caggattatg ataaccagca tgagcaagac 360

cgacctactc cgtcacctgc cccctctcgc cccttctcag ttcttcgtgc caatgatgtt 420

ttgtggcttt ccctcactgc cgctgagtat gaccagacta cgtatgggtc gtccaccaac 480

cctatgtatg tctctgacac agttacgctt gttaatgtgg ctactggtgc tcaggctgtt 540

gcccgctccc ttgattggtc taaagttact ctggacggcc gccccttac taccattcag 600

cagtattcta agacatttta tgttctcccg ctccgcggga agctgtcctt ttgggaggct 660

ggcacgacta aggccggcta cccttacaat tataatacta ccgctagtga ccaaattttg 720

attgagaatg cggccggcca ccgtgtcgct atttccacct ataccactag cttaggtgcc 780

ggtcctacct cgatctctgc ggtcggcgta ctggctccac actctgccct tgccgttctt 840

gaggatacta ttgattaccc cgcccgtgcc catacttttg atgatttttg cccggagtgc 900

cgtaccctag gtttgcaggg ttgtgcattc cagtctacta ttgctgagct ccagcgttta 960

aaaatgaagg taggtaaaac ccgggagtct gactacaagg acgacgatga caagtaataa 1020

ggatcc 1026

<210> 193
<211> 1389
<212> DNA
<213> Hepatitis E virus

<220>
<223> CKSORF32M-3.seq

<400> 193
gaattcatga ataacatgtc ttttgcatcg cccatgggat caccatgcgc cctagggctg 60

ttctgttgtt gctcttcgtg cttttgccta tgctgcccgc gccaccggcc agccagccgt 120

ctggccgccg tcgtgggcgg cgtagcggcg gtgccggcgg tggtttctgg ggtgacaggg 180

ttgattctca gcccttcgcc ctcccctata ttcatccaac caacccccttc gccgccgatg 240

tcgtttcaca acccggggct ggaactcgcc ctcgacagcc gccccgcccc cttggctccg 300

cttggcgtga ccagtcccag cgcccctccg ctgccccccg tcgtcgatct gccccagctt 360

ggtctgcgcc gcggtgctga tgggactgct gagcttacta ccacagcagc cacacgtttc 420

atgaaggacc tgcacttcgc tggcacgaat ggcgttggtg aggtgggtcg tggtatcgcc 480

ctgacactgt tcaatctcgc tgatacgctt ctcggcggtt taccgacaga attgatttcg 540

tcggctgggg gccaactgtt ttactcccgc ccggttgtct cagccaatgg cgagccaaca 600

gtaaagttat atacatctgt tgagaatgcg cagcaagaca agggcatcac cattccacat 660

gatatagacc tgggtgactc ccgtgtggtt atccaggatt atgataacca gcatgagcaa 720

gaccgaccta ctccgtcacc tgcccctct cgccccttct cagttcttcg tgccaatgat 780

gttttgtggc tttccctcac tgccgctgag tatgaccaga ctacgtatgg gtcgtccacc 840

aaccctatgt atgtctctga cacagttacg cttgttaatg tggctactgg tgctcaggct 900

gttgcccgct cccttgattg gtctaaagtt actctggacg gccgcccct tactaccatt 960

cagcagtatt ctaagacatt ttatgttctc ccgctccgcg ggaagctgtc cttttgggag 1020

gctggcacga ctaaggccgg ctacccttac aattataata ctaccgctag tgaccaaatt 1080

ttgattgaga atgcggccgg ccaccgtgtc gctatttcca cctataccac tagcttaggt 1140

gccggtccta cctcgatctc tgcggtcggc gtactggctc cacactctgc ccttgccgtt 1200

cttgaggata ctattgatta ccccgcccgt gcccatactt ttgatgattt ttgcccggag 1260

tgccgtaccc taggtttgca gggttgtgca ttccagtcta ctattgctga gctccagcgt 1320

ttaaaaatga aggtaggtaa aacccgggag tctgactaca aggacgacga tgacaagtaa 1380

taaggatcc 1389

<210> 194
<211> 408
<212> DNA
<213> Hepatitis E virus

<220>
<223> plorf3-12.con

<400> 194
gaattcatga ataacatgtc ttttgcatcg cccatgggat caccatgcgc cctagggctg 60

ttctgttgtt gctcttcgtg cttttgccta tgctgcccgc gccaccggcc ggccagccgt 120

ctggccgccg tcgtgggcgg cgcagcggcg gtgccggcgg tggtttctgg ggtgacaggg 180

ttgattctca gcccttcgcc ctcccctata ttcatccaac caacccccttc gccgccgatg 240

tcgtttcaca acccggggct ggaactcgcc ctcgacagcc gccccgcccc cttggctccg 300

cttggcgtga ccagtcccag cgcccctccg ctgccccccg tcgtcgatct gccccagctt 360

ggtctgcgcc gcgactacaa ggacgacgat gacaagtaat aaggatcc 408

<210> 195
<211> 1026
<212> DNA
<213> Hepatitis E virus

<220>
<223> plorf2.2-6.seq

<400> 195
gaattcatgg gtgctgatgg gactgctgag cttactacca cagcagccac acgtttcatg 60

aaggacctgc acttcgctgg cacgaatggc gttggtgagg tgggtcgtgg tatcgccctg 120

acactgttca atctcgctga tacgcttctc ggcggtttac cgacagaatt gatttcgtcg 180

gctgggggcc aactgtttta ctcccgcccg gttgtctcag ccaatggcga gccaacagta 240

aagttatata catctgttga gaatgcgcag caagacaagg gcatcaccat tccacatgat 300

atagacctgg gtgactcccg tgtggttatc caggattatg ataaccagca tgagcaagac 360

cgacctactc cgtcacctgc cccctctcgc cccttctcag ttcttcgtgc caatgatgtt 420

ttgtggcttt ccctcactgc cgctgagtat gaccagacta cgtatgggtc gtccaccaac 480

cctatgtatg tctctgacac agttacgctt gttaatgtgg ctactggtgc tcaggctgtt 540

gcccgctccc ttgattggtc taaagttact ctggacggcc gcccccttac taccattcag 600

cagtattcta agacatttta tgttctcccg ctccgcggga agctgtcctt ttgggaggct 660

ggcacgacta aggccggcta cccttacaat tataatacta ccgctagtga ccaaattttg 720

attgagaatg cggccggcca ccgtgtcgct atttccacct ataccactag cttaggtgcc 780

ggtcctacct cgatctctgc ggtcggcgta ctggctccac actctgccct tgccgttctt 840

gaggatacta ttgattaccc cgcccgtgcc catacttttg atgatttttg cccggagtgc 900

cgtaccctag gtttgcaggg ttgtgcattc cagtctacta ttgctgagct ccagcgttta 960

aaaatgaagg taggtaaaac ccgggagtct gactacaagg acgacgatga caagtaataa 1020

ggatcc 1026

<210> 196
<211> 1389
<212> DNA
<213> Hepatitis E virus

<220>
<223> PLORF32M-14-5.seq

<400> 196
gaattcatga ataacatgtc ttttgcatcg cccatgggat caccatgcgc cctagggctg 60

ttctgttgtt gctcttcgtg cttttgccta tgctgcccgc gccaccggcc agccagccgt 120

ctggccgccg tcgtgggcgg cgtagcggcg gtgccggcgg tggtttctgg ggtgacaggg 180

ttgattctca gcccttcgcc ctcccctata ttcatccaac caacccccttc gccgccgatg 240

tcgtttcaca acccggggct ggaactcgcc ctcgacagcc gccccgcccc cttggctccg 300

cttggcgtga ccagtcccag cgcccctccg ctgccccccg tcgtcgatct gccccagctt 360

ggtctgcgcc gcggtgctga tgggactgct gagcttacta ccacagcagc cacacgtttc 420

atgaaggacc tgcacttcgc tggcacgaat ggcgttggtg aggtgggtcg tggtatcgcc 480

ctgacactgt tcaatctcgc tgatacgctt ctcggcggtt taccgacaga attgatttcg 540

tcggctgggg gccaactgtt ttactcccgc ccggttgtct cagccaatgg cgagccaaca 600

gtaaagttat atacatctgt tgagaatgcg cagcaagaca agggcatcac cattccacat 660

gatatagacc tgggtgactc ccgtgtggtt atccaggatt atgataacca gcatgagcaa 720

gaccgaccta ctccgtcacc tgccccctct cgcccccttct cagttcttcg tgccaatgat 780

gttttgtggc tttccctcac tgccgctgag tatgaccaga ctacgtatgg gtcgtccacc 840

aaccctatgt atgtctctga cacagttacg cttgttaatg tggctactgg tgctcaggct 900

gttgcccgct cccttgattg gtctaaagtt actctggacg gccgcccccct tactaccatt 960

cagcagtatt ctaagacatt ttatgttctc ccgctccgcg ggaagctgtc cttttgggag 1020

gctggcacga ctaaggccgg ctacccttac aattataata ctaccgctag tgaccaaatt 1080

ttgattgaga atgcggccgg ccaccgtgtc gctatttcca cctataccac tagcttaggt 1140

gccggtccta cctcgatctc tgcggtcggc gtactggctc cacactctgc ccttgccgtt 1200

cttgaggata ctattgatta ccccgcccgt gcccatactt ttgatgattt ttgcccggag 1260

tgccgtaccc taggtttgca gggttgtgca ttccagtcta ctattgctga gctccagcgt 1320

ttaaaaatga aggtaggtaa aacccgggag tctgactaca aggacgacga tgacaagtaa 1380

taaggatcc 1389

<210> 197
<211> 74
<212> PRT
<213> Hepatitis E virus

<220>
<223> z12-orf3-5'.pep

<400> 197
Met Asn Asn Met Phe Cys Ala Ser Pro Met Gly Ser Pro Cys Ala Leu
1 5 10 15

Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys Pro Arg
20 25 30

```
His Arg Pro Ala Xaa Arg Leu Ala Ala Val Val Gly Gly Ala Ala Ala
         35                  40                  45

Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro Ser
     50                  55                  60

Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro
 65                  70
```

<210> 198
<211> 63
<212> DNA
<213> Hepatitis E virus

<220>
<223> Description of Artificial Sequence: Primer orf23p

<400> 198
tatatggatc cttattactt gtcatcgtcg tccttgtagt cagactcccg ggttttacct 60

acc 63

<210> 199
<211> 338
<212> PRT
<213> Hepatitis E virus

<220>
<223> cksorf2m-2.pep

<400> 199

```
Glu Phe Met Gly Ala Asp Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala
  1               5                  10                  15

Thr Arg Phe Met Lys Asp Leu His Phe Ala Gly Thr Asn Gly Val Gly
             20                  25                  30

Glu Val Gly Arg Gly Ile Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr
         35                  40                  45

Leu Leu Gly Gly Leu Pro Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln
     50                  55                  60

Leu Phe Tyr Ser Arg Pro Val Val Ser Ala Asn Gly Glu Pro Thr Val
 65                  70                  75                  80

Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln Gln Asp Lys Gly Ile Thr
                 85                  90                  95

Ile Pro His Asp Ile Asp Leu Gly Asp Ser Arg Val Val Ile Gln Asp
            100                 105                 110

Tyr Asp Asn Gln His Glu Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro
        115                 120                 125
```

```
Ser Arg Pro Phe Ser Val Leu Arg Ala Asn Asp Val Leu Trp Leu Ser
    130                 135                 140

Leu Thr Ala Ala Glu Tyr Asp Gln Thr Thr Tyr Gly Ser Ser Thr Asn
145                 150                 155                 160

Pro Met Tyr Val Ser Asp Thr Val Thr Leu Val Asn Val Ala Thr Gly
                165                 170                 175

Ala Gln Ala Val Ala Arg Ser Leu Asp Trp Ser Lys Val Thr Leu Asp
            180                 185                 190

Gly Arg Pro Leu Thr Thr Ile Gln Gln Tyr Ser Lys Thr Phe Tyr Val
        195                 200                 205

Leu Pro Leu Arg Gly Lys Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys
    210                 215                 220

Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu
225                 230                 235                 240

Ile Glu Asn Ala Ala Gly His Arg Val Ala Ile Ser Thr Tyr Thr Thr
                245                 250                 255

Ser Leu Gly Ala Gly Pro Thr Ser Ile Ser Ala Val Gly Val Leu Ala
            260                 265                 270

Pro His Ser Ala Leu Ala Val Leu Glu Asp Thr Ile Asp Tyr Pro Ala
        275                 280                 285

Arg Ala His Thr Phe Asp Asp Phe Cys Pro Glu Cys Arg Thr Leu Gly
    290                 295                 300

Leu Gln Gly Cys Ala Phe Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu
305                 310                 315                 320

Lys Met Lys Val Gly Lys Thr Arg Glu Ser Asp Tyr Lys Asp Asp Asp
                325                 330                 335

Asp Lys
```

<210> 200
<211> 338
<212> PRT
<213> Hepatitis E virus

<220>
<223> plorf2.2-6.pep

```
<400> 200
Glu Phe Met Gly Ala Asp Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala
  1               5                  10                  15

Thr Arg Phe Met Lys Asp Leu His Phe Ala Gly Thr Asn Gly Val Gly
             20                  25                  30
```

```
Glu Val Gly Arg Gly Ile Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr
          35                  40                  45

Leu Leu Gly Gly Leu Pro Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln
     50                  55                  60

Leu Phe Tyr Ser Arg Pro Val Val Ser Ala Asn Gly Glu Pro Thr Val
 65                  70                  75                  80

Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln Gln Asp Lys Gly Ile Thr
                 85                  90                  95

Ile Pro His Asp Ile Asp Leu Gly Asp Ser Arg Val Val Ile Gln Asp
            100                 105                 110

Tyr Asp Asn Gln His Glu Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro
        115                 120                 125

Ser Arg Pro Phe Ser Val Leu Arg Ala Asn Asp Val Leu Trp Leu Ser
    130                 135                 140

Leu Thr Ala Ala Glu Tyr Asp Gln Thr Thr Tyr Gly Ser Ser Thr Asn
145                 150                 155                 160

Pro Met Tyr Val Ser Asp Thr Val Thr Leu Val Asn Val Ala Thr Gly
                165                 170                 175

Ala Gln Ala Val Ala Arg Ser Leu Asp Trp Ser Lys Val Thr Leu Asp
            180                 185                 190

Gly Arg Pro Leu Thr Thr Ile Gln Gln Tyr Ser Lys Thr Phe Tyr Val
        195                 200                 205

Leu Pro Leu Arg Gly Lys Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys
    210                 215                 220

Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu
225                 230                 235                 240

Ile Glu Asn Ala Ala Gly His Arg Val Ala Ile Ser Thr Tyr Thr Thr
                245                 250                 255

Ser Leu Gly Ala Gly Pro Thr Ser Ile Ser Ala Val Gly Val Leu Ala
            260                 265                 270

Pro His Ser Ala Leu Ala Val Leu Glu Asp Thr Ile Asp Tyr Pro Ala
        275                 280                 285

Arg Ala His Thr Phe Asp Asp Phe Cys Pro Glu Cys Arg Thr Leu Gly
    290                 295                 300

Leu Gln Gly Cys Ala Phe Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu
305                 310                 315                 320

Lys Met Lys Val Gly Lys Thr Arg Glu Ser Asp Tyr Lys Asp Asp Asp
                325                 330                 335
```

Asp Lys

<210> 201
<211> 37
<212> DNA
<213> Hepatitis E virus

<220>
<223> Description of Artificial Sequence: Primer orf35p

<400> 201
tatatgaatt catgaataac atgtcttttg catcgcc 37

<210> 202
<211> 68
<212> DNA
<213> Hepatitis E virus

<220>
<223> Description of Artificial Sequence: Primer orf33p

<400> 202
tatatggatc cttattactt gtcatcgtcg tccttgtagt cgcggcgcag accaagctgg 60

ggcagatc 68

<210> 203
<211> 132
<212> PRT
<213> Hepatitis E virus

<220>
<223> pJOorf3-29.pep

<400> 203
Glu Phe Met Asn Asn Met Ser Phe Ala Ser Pro Met Gly Ser Pro Cys
1 5 10 15

Ala Leu Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys
20 25 30

Pro Arg His Arg Pro Ala Ser Arg Leu Ala Ala Val Val Gly Gly Ala
35 40 45

Ala Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser
50 55 60

Pro Ser Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met
65 70 75 80

Ser Phe His Asn Pro Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala
85 90 95

```
Pro Leu Ala Pro Leu Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Pro
            100                 105                 110

Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg Arg Asp Tyr Lys Asp
        115                 120                 125

Asp Asp Asp Lys
    130


<210> 204
<211> 132
<212> PRT
<213> Hepatitis E virus

<220>
<223> plorf3-12.pep

<400> 204
Glu Phe Met Asn Asn Met Ser Phe Ala Ser Pro Met Gly Ser Pro Cys
  1               5                  10                  15

Ala Leu Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys
             20                  25                  30

Pro Arg His Arg Pro Ala Ser Arg Leu Ala Ala Val Val Gly Gly Ala
         35                  40                  45

Ala Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser
     50                  55                  60

Pro Ser Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met
 65                  70                  75                  80

Ser Phe His Asn Pro Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala
                 85                  90                  95

Pro Leu Ala Pro Leu Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Pro
            100                 105                 110

Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg Arg Asp Tyr Lys Asp
        115                 120                 125

Asp Asp Asp Lys
    130


<210> 205
<211> 48
<212> DNA
<213> Hepatitis E virus

<220>
<223> Description of Artificial Sequence: Primer orf23

<400> 205
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ctcagcagtc ccatcagcac cgcggcgcag accaagctgg ggcagatc 48

<210> 206
<211> 459
<212> PRT
<213> Hepatitis E virus

<220>
<223> CKSORF32M-3.pep

<400> 206

```
Glu Phe Met Asn Asn Met Ser Phe Ala Ser Pro Met Gly Ser Pro Cys
  1               5                  10                  15

Ala Leu Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys
             20                  25                  30

Pro Arg His Arg Pro Ala Ser Arg Leu Ala Ala Val Val Gly Gly Val
         35                  40                  45

Ala Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser
     50                  55                  60

Pro Ser Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met
 65                  70                  75                  80

Ser Phe His Asn Pro Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala
                 85                  90                  95

Pro Leu Ala Pro Leu Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Pro
            100                 105                 110

Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg Arg Gly Ala Asp Gly
        115                 120                 125

Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu
    130                 135                 140

His Phe Ala Gly Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile Ala
145                 150                 155                 160

Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr
                165                 170                 175

Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val
            180                 185                 190

Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu
        195                 200                 205

Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp Leu
    210                 215                 220

Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln
225                 230                 235                 240
```

```
Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu
                245                 250                 255

Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp
            260                 265                 270

Gln Thr Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp Thr
        275                 280                 285

Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser
    290                 295                 300

Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr Ile
305                 310                 315                 320

Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys Leu
                325                 330                 335

Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr
            340                 345                 350

Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly His
        355                 360                 365

Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Thr
    370                 375                 380

Ser Ile Ser Ala Val Gly Val Leu Ala Pro His Ser Ala Leu Ala Val
385                 390                 395                 400

Leu Glu Asp Thr Ile Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp
                405                 410                 415

Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln
            420                 425                 430

Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr
        435                 440                 445

Arg Glu Ser Asp Tyr Lys Asp Asp Asp Asp Lys
    450                 455


<210> 207
<211> 459
<212> PRT
<213> Hepatitis E virus

<220>
<223> PLORF32M-14-5.pep

<400> 207
Glu Phe Met Asn Asn Met Ser Phe Ala Ser Pro Met Gly Ser Pro Cys
  1               5                  10                  15

Ala Leu Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys
             20                  25                  30
```

```
Pro Arg His Arg Pro Ala Ser Arg Leu Ala Ala Val Val Gly Gly Val
         35                  40                  45

Ala Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser
     50                  55                  60

Pro Ser Pro Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met
 65                  70                  75                  80

Ser Phe His Asn Pro Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala
                 85                  90                  95

Pro Leu Ala Pro Leu Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Pro
            100                 105                 110

Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg Arg Gly Ala Asp Gly
        115                 120                 125

Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu
    130                 135                 140

His Phe Ala Gly Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile Ala
145                 150                 155                 160

Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr
                165                 170                 175

Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val
            180                 185                 190

Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu
        195                 200                 205

Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp Leu
    210                 215                 220

Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln
225                 230                 235                 240

Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu
                245                 250                 255

Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp
            260                 265                 270

Gln Thr Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp Thr
        275                 280                 285

Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser
    290                 295                 300

Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr Ile
305                 310                 315                 320

Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys Leu
                325                 330                 335
```

```
Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr
            340                 345                 350

Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly His
        355                 360                 365

Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Thr
    370                 375                 380

Ser Ile Ser Ala Val Gly Val Leu Ala Pro His Ser Ala Leu Ala Val
385                 390                 395                 400

Leu Glu Asp Thr Ile Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp
                405                 410                 415

Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln
            420                 425                 430

Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr
        435                 440                 445

Arg Glu Ser Asp Tyr Lys Asp Asp Asp Asp Lys
    450                 455


<210> 208
<211> 36
<212> DNA
<213> Hepatitis E virus

<220>
<223> Description of Artificial Sequence: Primer
      orf2mid5p

<400> 208
tatatgaatt catgggtgct gatgggactg ctgagc                                  36


<210> 209
<211> 418
<212> DNA
<213> Hepatitis E virus

<220>
<223> 1440o1.seq

<220>
<221> CDS
<222> (3)..(416)

<400> 209
ct ggc aty act act gcy att gag cag gct gct ctg gct gcg gcc aat    47
   Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
     1               5                  10                  15

tcc gcc ttg gcg aat gct gtg gtg gtt cgg ccg ttt tta tcc cgt gtt    95
```

```
Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val
                 20                  25                  30

caa act gat atc ctt att aac ctg atg caa ccc cgt cag ctt gtg ttc   143
Gln Thr Asp Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe
            35                  40                  45

cgg cct gaa gtt ctc tgg aac cat ccg atc cag cga gtt ata cat aat   191
Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
        50                  55                  60

gag ctg gaa caa tac tgt cga gcc cgc gct ggc cgc tgt ctt gag gtg   239
Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val
    65                  70                  75

ggc gct cac cca agg tct att aat gat aac ccc aat gtt ctg cac cgg   287
Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg
80                  85                  90                  95

tgc ttt ctc cgc ccg gtt ggg aga gac gtc cag cgc tgg tat tcc gcc   335
Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
                100                 105                 110

ccc act cgt ggt cca gcg gct aac tgc cgc cgt tct gcg cta cgc ggt   383
Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
            115                 120                 125

ttg ccc cct gtc gac cgc act tac tgt yty gat gg                   418
Leu Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
        130                 135


<210> 210
<211> 138
<212> PRT
<213> Hepatitis E virus

<400> 210
Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn Ser
  1               5                  10                  15

Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val Gln
            20                  25                  30

Thr Asp Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe Arg
        35                  40                  45

Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn Glu
    50                  55                  60

Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val Gly
65                  70                  75                  80

Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg Cys
                85                  90                  95

Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro
```

```
            100                 105                 110

Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu
        115                 120                 125

Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
    130                 135


<210> 211
<211> 197
<212> DNA
<213> Hepatitis E virus

<220>
<223> 1440o?.seq

<220>
<221> CDS
<222> (2)..(196)

<400> 211
g aca gaa ttr att tcg tcg gct gga ggt caa ctg ttc tac tcc cgc ccg 49
  Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
    1               5                  10                  15

gtt gtc tca gcc aat ggc gag ccg act gtt aag tta tac acc tct gtc   97
Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
            20                  25                  30

gag aat gca cag cag gat aag ggc att gct ata cca cat gat ata gac   145
Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35                  40                  45

tta ggg gat tcc cgt gtg gtt ata caa gat tat gay aac car cay gaa   193
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50                  55                  60

caa g                                                             197
Gln
 65


<210> 212
<211> 65
<212> PRT
<213> Hepatitis E virus

<400> 212
Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
  1               5                  10                  15

Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
            20                  25                  30

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35                  40                  45
```

```
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50                  55                  60

Gln
 65


<210> 213
<211> 418
<212> DNA
<213> Hepatitis E virus

<220>
<223> 2015-1.seq

<220>
<221> CDS
<222> (3)..(416)

<400> 213
ct ggc aty act act gcy att gag cag gct gct ctg gct gcg gct aac    47
   Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
     1               5                  10                  15

tct gcc ttg gcg aat gct gtg gtg gtc cgg ccg ttc ctg tcc cgc act   95
Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Thr
                 20                  25                  30

cag act gat att ctt att aat ttg atg caa ccc cgg caa ctt gta ttc   143
Gln Thr Asp Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe
             35                  40                  45

cgc cct gag gtt ttg tgg aac cat ccg atc cag cga gtc ata cat aat   191
Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
         50                  55                  60

gag ctg gag cag tat tgc cgt gct cgt gct ggt cgc tgc ctg gag gtt   239
Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val
     65                  70                  75

ggg gct cat cca aga tct atc aat gac aac cct aat gtt ctg cac cgg   287
Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg
 80                  85                  90                  95

tgt ttc ctc cgt ccg gtt ggg cga gac gta cag cgt tgg tat tct gcc   335
Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
                100                 105                 110

cct act cgc ggc ccg gcg gct aat tgc cgc cgt tcc gcg tta cgt ggc   383
Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
            115                 120                 125

cta cct cct gtc gac cgc act tac tgt yty gat gg                    418
Leu Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
        130                 135
```

<210> 214
<211> 138
<212> PRT
<213> Hepatitis E virus

<400> 214
Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn Ser
1 5 10 15

Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Thr Gln
20 25 30

Thr Asp Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe Arg
35 40 45

Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn Glu
50 55 60

Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val Gly
65 70 75 80

Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg Cys
85 90 95

Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro
100 105 110

Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu
115 120 125

Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
130 135

<210> 215
<211> 197
<212> DNA
<213> Hepatitis E virus

<220>
<223> 2015o2.seq

<220>
<221> CDS
<222> (2)..(196)

<400> 215
g aca gaa ttr att tcg tcg gct gga ggc cag ctc ttc tac tcc cgc cca 49
Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
1 5 10 15

gtc gtc tca gcc aat ggc gag ccg act gtt aaa ttg tat aca tcc gtc 97
Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
20 25 30

gag aat gcg cag cag gac aag ggc att gcc ata cca cat gat ata gat 145

```
Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35                  40                  45

cta gga gat tcc cgc gtg gtt atc cag gat tat gay aac car cay gaa   193
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50                  55                  60

caa g                                                             197
Gln
 65


<210> 216
<211> 65
<212> PRT
<213> Hepatitis E virus

<400> 216
Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
  1               5                  10                  15

Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
             20                  25                  30

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35                  40                  45

Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50                  55                  60

Gln
 65


<210> 217
<211> 251
<212> DNA
<213> Hepatitis E virus

<220>
<223> 14404-2.seq

<220>
<221> CDS
<222> (3)..(251)
<223> orf2

<220>
<223> orf3 from position 1 to position 165

<400> 217
at att cat cca acc aac ccc ttt gcc tcc gac gtc gta tcg caa tcc    47
   Ile His Pro Thr Asn Pro Phe Ala Ser Asp Val Val Ser Gln Ser
     1               5                  10                  15

ggg gct gga gct cgc cct cga cag ccg gcc cgc ccc ctc ggc tcc tct   95
Gly Ala Gly Ala Arg Pro Arg Gln Pro Ala Arg Pro Leu Gly Ser Ser
```

```
                  20                  25                  30

tgg cgt gac cag tcc cag cgc ccc ccc gct gtc ccc cgt cgt cga tct   143
Trp Arg Asp Gln Ser Gln Arg Pro Pro Ala Val Pro Arg Arg Arg Ser
            35                  40                  45

acc cca act ggg gct gcg ccg cta act gct gtt tca cca gcg cct gat   191
Thr Pro Thr Gly Ala Ala Pro Leu Thr Ala Val Ser Pro Ala Pro Asp
        50                  55                  60

acg gcc cca gtc cct gat gtt gac tct cgt ggc gct atc ttg cgc cgg   239
Thr Ala Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu Arg Arg
    65                  70                  75

cag tat aac cta                                                  251
Gln Tyr Asn Leu
 80
```

<210> 218
<211> 83
<212> PRT
<213> Hepatitis E virus

<400> 218

```
Ile His Pro Thr Asn Pro Phe Ala Ser Asp Val Val Ser Gln Ser Gly
  1               5                  10                  15

Ala Gly Ala Arg Pro Arg Gln Pro Ala Arg Pro Leu Gly Ser Ser Trp
            20                  25                  30

Arg Asp Gln Ser Gln Arg Pro Pro Ala Val Pro Arg Arg Arg Ser Thr
        35                  40                  45

Pro Thr Gly Ala Ala Pro Leu Thr Ala Val Ser Pro Ala Pro Asp Thr
    50                  55                  60

Ala Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu Arg Arg Gln
65                  70                  75                  80

Tyr Asn Leu
```

<210> 219
<211> 55
<212> PRT
<213> Hepatitis E virus

<220>
<223> 14404-2.seq orf3

<400> 219

```
Ile Phe Ile Gln Pro Thr Pro Leu Pro Pro Thr Ser Tyr Arg Asn Pro
  1               5                  10                  15

Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala Pro Ser Ala Pro Leu
```

```
            20                  25                  30

Gly Val Thr Ser Pro Ser Ala Pro Pro Leu Ser Pro Val Val Asp Leu
        35                  40                  45

Pro Gln Leu Gly Leu Arg Arg
    50                  55


<210> 220
<211> 251
<212> DNA
<213> Hepatitis E virus

<220>
<223> 20154-2.seq

<220>
<221> CDS
<222> (3)..(251)
<223> orf2

<220>
<223> orf3 from position 1 to position 165

<400> 220
at att cat cca acc aac ccc ttt gcc gcc gac gtc gta tca caa ccc    47
   Ile His Pro Thr Asn Pro Phe Ala Ala Asp Val Val Ser Gln Pro
     1               5                  10                  15

ggg gct gga gct cgc cct cga cag ccg ccc cgc ccc ctc ggc tcc tct   95
Gly Ala Gly Ala Arg Pro Arg Gln Pro Pro Arg Pro Leu Gly Ser Ser
                 20                  25                  30

tgg cgt gat cag tcc cag cgc ccc tcc gct gcc ccc cgt cgt cga tct   143
Trp Arg Asp Gln Ser Gln Arg Pro Ser Ala Ala Pro Arg Arg Arg Ser
             35                  40                  45

acc cca gct ggg gct gcg ccg tta act gct gtt tcc cct gcg ccc gat   191
Thr Pro Ala Gly Ala Ala Pro Leu Thr Ala Val Ser Pro Ala Pro Asp
         50                  55                  60

acg gcc cca gtc ccc gac gtt gat tcc cgt ggt gcc atc ctg cgc cgg   239
Thr Ala Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu Arg Arg
     65                  70                  75

cag tat aac cta                                                  251
Gln Tyr Asn Leu
 80


<210> 221
<211> 83
<212> PRT
<213> Hepatitis E virus

<400> 221
```

Ile His Pro Thr Asn Pro Phe Ala Ala Asp Val Val Ser Gln Pro Gly
1 5 10 15

Ala Gly Ala Arg Pro Arg Gln Pro Pro Arg Pro Leu Gly Ser Ser Trp
20 25 30

Arg Asp Gln Ser Gln Arg Pro Ser Ala Ala Pro Arg Arg Arg Ser Thr
35 40 45

Pro Ala Gly Ala Ala Pro Leu Thr Ala Val Ser Pro Ala Pro Asp Thr
50 55 60

Ala Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu Arg Arg Gln
65 70 75 80

Tyr Asn Leu

<210> 222
<211> 55
<212> PRT
<213> Hepatitis E virus

<220>
<223> 20154-2.seq orf3

<400> 222
Ile Phe Ile Gln Pro Thr Pro Leu Pro Pro Thr Ser Tyr His Asn Pro
1 5 10 15

Gly Leu Glu Leu Ala Leu Asp Ser Arg Pro Ala Pro Ser Ala Pro Leu
20 25 30

Gly Val Ile Ser Pro Ser Ala Pro Pro Leu Pro Pro Val Val Asp Leu
35 40 45

Pro Gln Leu Gly Leu Arg Arg
50 55

<210> 223
<211> 48
<212> PRT
<213> Hepatitis E virus

<220>
<223> US-2 3-2e

<400> 223
Thr Ile Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro
1 5 10 15

Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Ile
20 25 30

Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu Ser

35 40 45

<210> 224
<211> 33
<212> PRT
<213> Hepatitis E virus

<220>
<223> US-2 4-2

<400> 224
Asp Ser Arg Pro Ala Pro Leu Val Pro Leu Gly Val Thr Ser Pro Ser
1 5 10 15

Ala Pro Pro Leu Pro Pro Val Val Asp Leu Pro Gln Leu Gly Leu Arg
20 25 30

Arg

<210> 225
<211> 450
<212> DNA
<213> Hepatitis E virus

<220>
<223> 5p.pile {hpesvp}

<400> 225
ggctcctggc atcactactg ctattgagca ggctgctcta gcagcggcca actctgccct 60

ggcgaatgct gtggtagtta ggccttttct ctctcaccag cagattgaga tcctcattaa 120

cctaatgcaa cctcgccagc ttgttttccg ccccgaggtt ttctggaatc atcccatcca 180

gcgtgtcatc cataacgagc tggagcttta ctgccgcgcc cgctccggcc gctgtcttga 240

aattggcgcc catccccgct caataaatga taatcctaat gtggtccacc gctgcttcct 300

ccgccctgtt gggcgtgatg ttcagcgctg gtatactgct cccactcgcg ggccggctgc 360

taattgccgg cgttccgcgc tgcgcgggct tcccgctgct gaccgcactt actgcctcga 420

cgggttttct ggctgtaact ttcccgccga 450

<210> 226
<211> 450
<212> DNA
<213> Hepatitis E virus

<220>

<223> 5p.pile {hpeuigh}

<400> 226
ggctcctggc atcactactg ctattgagca ggctgctcta gcagcggcca attctgccct 60

tgcgaatgct gtggtagtta ggccttttct ctctcaccag cagattgaga tccttattaa 120

cctaatgcaa cctcgccagc ttgttttccg ccccgaggtt ttctggaacc accccatcca 180

gcgtgtcatc cataatgagc tggagcttta ctgtcgcgcc cgctccggcc gctgccttga 240

aattggtgcc caccctcgct caataaacga caatcctaat gtggtccacc gctgcttcct 300

ccgccctgcc gggcgtgatg ttcagcgttg gtatactgct cctacccgcg ggccggctgc 360

taattgccgg ggttccgcac tgcgcgggct ccccgctgct gaccgcactt actgcttcga 420

cgggttttct ggctgtaact ttcccgccga 450

<210> 227
<211> 450
<212> DNA
<213> Hepatitis E virus

<220>
<223> 5p.pile {hpea}

<400> 227
ggctcctggc atcactactg ctattgagca ggctgctcta gcagcggcca actctgccct 60

tgcgaatgct gtggtagtta ggccttttct ctctcaccag cagattgaga tccttattaa 120

cctaatgcaa cctcgccagc ttgttttccg ccccgaggtt ttctggaacc atcccatcca 180

gcgtgttatc cataatgagc tggagcttta ctgtcgcgcc cgctccggcc gctgcctcga 240

aattggtgcc caccccgct caataaatga caatcctaat gtggtccacc gttgcttcct 300

ccgtcctgcc gggcgtgatg ttcagcgttg gtatactgcc cctacccgcg ggccggctgc 360

taattgccgg cgttccgcgc tgcgcgggct ccccgctgct gaccgcactt actgcttcga 420

cgggttttct ggctgtaact ttcccgccga 450

<210> 228
<211> 446
<212> DNA
<213> Hepatitis E virus

<220>
<223> 5p.pile {840455p}

<400> 228
cctggcatta ctactgccat tgagcaggct gctctggctg cggccaattc tgccttggcg 60

aatgctgtgg tggttcggcc gtttttatct cgcgtgcaaa ccgagattct tattaatttg 120

atgcaacccc ggcagttggt tttccgccct gaggtacttt ggaatcaccc tatccagcgg 180

gttatacata atgaattaga acagtactgc cgggctcggg ctggtcgttg cttggaggtt 240

ggagctcacc caagatccat taatgacaac cccaacgttc tgcatcggtg tttccttaga 300

ccggttggcc gagatgttca gcgctggtac tctgccccca cccgcggccc tgcggctaat 360

tgccgccgct ccgcgttgcg tggtctcccc cccgctgacc gcacttactg ctttgatgga 420

ttctcccgtt gtgcttttgc tgcaga 446

<210> 229
<211> 450
<212> DNA
<213> Hepatitis E virus

<220>
<223> 5p.pile {hpenssp}

<400> 229
ggctcctggc atcactactg ctattgagca agcagctcta gcagcggcca actccgccct 60

tgcgaatgct gtggtggtcc ggcctttcct ttcccatcag caggttgaga tccttataaa 120

tctcatgcaa cctcggcagc tggtgtttcg tcctgaggtt ttttggaatc acccgattca 180

acgtgttata cataatgagc ttgagcagta ttgccgtgct cgctcgggtc gctgccttga 240

gattggagcc cacccacgct ccattaatga taatcctaat gtcctccatc gctgctttct 300

ccaccccgtc ggccgggatg ttcagcgctg gtacacagcc ccgactaggg gacctgcggc 360

gaactgtcgc cgctcggcac ttcgtggtct gccaccagcc gaccgcactt actgttttga 420

tggctttgcc ggctgccgtt ttgccgccga 450

<210> 230
<211> 450
<212> DNA
<213> Hepatitis E virus

<220>
<223> 5p Consensus

<220>
<221> variation
<222> ()..(450)
<223> The nucleotide identity of each n is indicated in
Figure 9.

<400> 230
nnnncctggc atnactactg cnattgagca ngcngctctn gcngcggcca antcngccnt 60

ngcgaatgct gtggtngtnn ggccnttnnt ntcncnnnng cannnngaga tnctnatnaa 120

nntnatgcaa ccncgncagn tngtnttncg nccngaggtn ntntggaanc anccnatnca 180

ncgngtnatn cataangann tngancnnta ntgncgngcn cgnncnggnc gntgnntnga 240

nnttggngcn canccnngnt cnatnaanga naanccnaan gtnntncanc gntgnttnct 300

nnnnccngnn ggncgngatg ttcagcgntg gtanncngcn ccnacnngng gnccngcngc 360

naantgncgn ngntcngcnn tncgnggnct nccnncngcn gaccgcactt actgnntnga 420

nggnttnncn ngntgnnnnt ttncngcnga 450

<210> 231
<211> 300
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpea} shown in Figure 9B

<400> 231
actgagtcag tgaagccagt gcttgacctg acaaattcaa ttctgtgtcg ggtggaatga 60

ataacatgtc ttttgctgcg cccatgggtt cgcgaccatg cgccctcggc ctattttgct 120

gttgctcctc atgtttctgc ctatgctgcc cgcgccaccg cccggtcagc cgtctggccg 180

ccgtcgtggg cggcgcagcg gcggttccgg cggtggtttc tggggtgacc gggttgattc 240

tcagcccttc gcaatcccct atattcatcc aaccaacccc ttcgcccccg atgtcaccgc 300

<210> 232
<211> 300
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpeuigh} shown in Figure 9B

<400> 232
actgagtcgg tgaagccagt gctcgacttg acaaattcaa tcctgtgtcg ggtggaatga 60

ataacatgtc ttttgctgcg cccatgggtt ggcgaccatg cgccctcggc ctattttgct 120

gttgctcctc atgtttctgc ctatcgtgcc cgcgccaccg cccggtcagc cgtctggccg 180

ccgtcgtggg cggcgcagcg gcggttccgg cggtggtttc tggggtgacc gggttgattc 240

tcagcccttc gcaatcccct atattcatcc aaccaacccc ttcgcccccg atgtcaccgc 300

<210> 233

<211> 300
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpesvp} shown in Figure 9B

<400> 233
actgagtcag taaaaccagt gctcgacttg acaaattcaa tcttgtgtcg ggtggaatga 60

ataacatgtc ttttgctgcg cccatgggtt cgcgaccatg cgccctcggc ctattttgtt 120

gctgctcctc atgtttttgc ctatgctgcc cgcgccaccg cccggtcagc cgtctggccg 180

ccgtcgtggg cggcgcagcg gcggttccgg cggtggtttc tggggtgacc gggttgattc 240

tcagcccttc gcaatcccct atattcatcc aaccaacccc ttcgcccccg atgtcaccgc 300

<210> 234
<211> 300
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpenssp} shown in Figure 9B

<400> 234
acagagtctg ttaagcctat acttgacctt acacactcaa ttatgcaccg gtctgaatga 60

ataacatgtg gtttgctgcg cccatgggtt cgccaccatg cgccctaggc ctcttttgct 120

gttgttcctc ttgtttctgc ctatgttgcc cgcgccaccg accggtcagc cgtctggccg 180

ccgtcgtggg cggcgcagcg gcggtaccgg cggtggtttc tggggtgacc gggttgattc 240

tcagcccttc gcaatcccct atattcatcc aaccaacccc tttgccccag acgttgccgc 300

<210> 235
<211> 297
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {840453p} shown in Figure 9B

<400> 235
acagagacta ttaaacctgt acttgatctc acaaattcca tcatacagcg ggtggaatga 60

ataacatgtc ttttgcatcg cccatgggat caccatgcgc cctagggctg ttctgttgtt 120

gttcctcatg tttctgccta tgctgcccgc gccaccggcc ggtcagccgt ctggccgtcg 180

ccgtgggcgg cgcagcggcg gtgccggcgg tggtttctgg agtgacaggg ttgattctca 240

gcccttcgcc ctcccctata ttcatccaac caacccccttc gccgccgatg tcgtttc 297

<210> 236
<211> 300
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p Consensus shown in Figure 9B

<220>
<221> variation
<222> (1)..(300)
<223> The nucleotide identity of each n is indicated in
Figure 9B

<400> 236
acngagncnn tnaanccnnt nctnganntn acanantcna tnntnnnncg gnnngaatga 60

ataacatgtn ntttgcnncg cccatgggnt nnnnaccatg cgccctnggn ctnttntgnt 120

gntgntcctc ntgtttntgc ctatnntgcc cgcgccaccg nccggtcagc cgtctggccg 180

ncgncgtggg cggcgcagcg gcggtnccgg cggtggtttc tggngtgacn gggttgattc 240

tcagcccttc gcnntcccct atattcatcc aaccaacccc ttngccncng angtnnnnnc 300

<210> 237
<211> 250
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpea} shown in Figure 9C

<400> 237
agcgcttacc ctgtttaacc ttgctgacac cctgcttggc ggtctaccga cagaattgat 60

ttcgtcggct ggtggccagc tgttctactc tcgccccgtc gtctcagcca atggcgagcc 120

gactgttaag ctgtatacat ctgtggagaa tgctcagcag gataagggta ttgcaatccc 180

gcatgacatc gacctcgggg aatcccgtgt agttattcag gattatgaca accaacatga 240

gcaggaccga 250

<210> 238
<211> 250
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpeuigh} shown in Figure 9C

<400> 238

agcgcttacc ctgtttaacc ttgctgacac cctgcttggc ggtctaccga cagaattgat 60

ttcgtcggct ggtggccagc tgttctactc tcgccccgtc gtctcagcca atggcgagcc 120

gactgttaag ctgtatacat ctgtagagaa tgctcagcag gataagggta ttgcaatccc 180

gcatgacatc gacctcgggg aatctcgagt tgttattcag gattatgaca accaacatga 240

gcaggaccgg 250

<210> 239
<211> 250
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpesvp} shown in Figure 9C

<400> 239
agccctcacc ctgttcaacc ttgctgacac tctgcttggc ggcctgccga cagaattgat 60

ttcgtcggct ggtggccagc tgttctactc ccgtcccgtt gtctcagcca atggcgagcc 120

gactgttaag ttgtatacat ctgtagagaa tgctcagcag gataagggta ttgcaatccc 180

gcatgacatt gacctcggag aatctcgtgt ggttattcag gattatgata accaacatga 240

acaagatcgg 250

<210> 240
<211> 250
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p.pile {hpenssp} shown in Figure 9C

<400> 240
agctctaaca ttacttaacc ttgctgacac gctcctcggc gggctcccga cagaattaat 60

ttcgtcggct ggcgggcaac tgttttattc ccgcccggtt gtctcagcca atggcgagcc 120

aaccgtgaag ctctatacat cagtggagaa tgctcagcag gataagggtg ttgctatccc 180

ccacgatatc gatcttggtg attcgcgtgt ggtcattcag gattatgaca accagcatga 240

gcaggatcgg 250

<210> 241
<211> 250
<212> DNA
<213> Hepatitis E virus

<220>

<223> 3p.pile {840453p} shown in Figure 9C

<400> 241
tgccctgact ctgtttaatc ttgctgatac gcttcttggt ggtttaccga cagaattgat 60

ttcgtcggct gggggtcaac tgttttactc ccgccctgtt cagaattgat ttcgtcggct 120

gggggtcaac tgttttactc ccgccctgtt tgcgcagcaa gacaagggca tcaccattcc 180

acacgacata gatttaggtg actcccgtgt ggttatccag gattatgata accagcacga 240

acaagatcga 250

<210> 242
<211> 250
<212> DNA
<213> Hepatitis E virus

<220>
<223> 3p Consensus shown in Figure 9C

<220>
<221> variation
<222> ()..(250)
<223> The nucleotide identity of each n is indicated in
Figure 9C

<400> 242
ngcnctnacn ntnntnaanc ttgctganac nctnctnggn ggnntnccga cagaattnat 60

ttcgtcggct ggnggncanc tgttntantc ncgnccngtn gtctcngcca atggcgagcc 120

nacngtnaag ntntanacat cngtngagaa tgcncagcan ganaacgggnn tnncnatncc 180

ncanganatn ganntnggng antcncgngt ngtnatncag gattatgana accancanga 240

ncangancgn 250

<210> 243
<211> 418
<212> DNA
<213> Hepatitis E virus

<220>
<223> Aulol-wlabolpl.pat

<220>
<221> CDS
<222> (3)..(416)

<400> 243
ct ggc aty act act gcy att gag caa gct gct ctg gct gcg gcc aat 47
Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
1 5 10 15

```
tct gcc ttg gcg aat gct gtg gtg gtt cgg ccg ttt tta tcc cgt gtg    95
Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val
                 20                  25                  30

cag act gag atc ctt att aac ttg atg caa cct cgg cag ctg gtg ttc   143
Gln Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe
             35                  40                  45

cga cct gag gtg ctt tgg aat cat ccc att cag cgg gtt atc cat aat   191
Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
         50                  55                  60

gag tta gaa caa tac tgc cgg gcc cgg gcc ggc cgt tgc cta gag gtg   239
Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val
     65                  70                  75

ggg gcc cac cca agg tcc att aac gat aac ccc aat gtt ttg cac cgg   287
Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg
 80                  85                  90                  95

tgt ttt ctg cga ccg gtc ggg agg gat gtt cag cgc tgg tac tct gcc   335
Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
                100                 105                 110

ccc acc cgc ggc cct gcg gct aac tgc cgc cgc tcc gct ttg cgt ggc   383
Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
            115                 120                 125

ctt ccc ccc gtc gac cgc act tac tgt yty gat gg                    418
Leu Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
        130                 135
```

<210> 244
<211> 138
<212> PRT
<213> Hepatitis E virus

<400> 244

```
Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn Ser
  1               5                  10                  15

Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val Gln
             20                  25                  30

Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe Arg
         35                  40                  45

Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn Glu
     50                  55                  60

Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val Gly
 65                  70                  75                  80

Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg Cys
                 85                  90                  95
```

```
Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro
            100                 105                 110

Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu
        115                 120                 125

Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
    130                 135


<210> 245
<211> 197
<212> DNA
<213> Hepatitis E virus

<220>
<223> Aulo2-wlao2.pat

<220>
<221> CDS
<222> (2)..(196)

<400> 245
g aca gaa ttr att tcg tcg gct ggg gga cag tta ttc tac tcc cgc cct 49
  Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
    1               5                  10                  15

gty gtc tca gcc aat ggc gag ccg act gtt aaa tta tat aca tct gta   97
Xaa Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
             20                  25                  30

gag aat gcg cag cag gac aag ggg att gcc atc cca cat gat ata gat   145
Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35                  40                  45

ctg ggc gac tct cgt gtg gtg atc cag gat tat gay aac car cay gaa   193
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50                  55                  60

caa g                                                            197
Gln
 65


<210> 246
<211> 65
<212> PRT
<213> Hepatitis E virus

<400> 246
Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
  1               5                  10                  15

Xaa Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
             20                  25                  30

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
```

```
         35                  40                  45

Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50                  55                  60

Gln
 65
```

<210> 247
<211> 418
<212> DNA
<213> Hepatitis E virus

<220>
<223> Arlol- f73olpl.pat

<220>
<221> CDS
<222> (3)..(416)

<400> 247

```
ct ggc aty act act gcy att gag caa gct gct ctg gct gcg gcc aac    47
   Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
     1               5                  10                  15

tct gcc ttg gcg aat gct gtg gtg gtt cgg ccg ttt tta tcc cgt gtg   95
Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val
                 20                  25                  30

cag acc gag att ctt att aac cta atg caa ccc cgg cag ctg gtt ttt   143
Gln Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe
             35                  40                  45

cgt cct gag gtg ctt tgg aac cat cct atc cag cgg gtt att cat aat   191
Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
         50                  55                  60

gag tta gaa cag tac tgt cgg gct cgg gct ggt cgc tgc cta gag gtc   239
Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val
     65                  70                  75

ggg gcc cac cca agg tcc att aat gat aac cct aat gtt ttg cac cgg   287
Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg
 80                  85                  90                  95

tgc ttc cta cga cca gtc ggg agg gat gtt caa cgt tgg tat tcc gcc   335
Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
                100                 105                 110

ccc acc cgc ggt cct gct gcc aac tgc cgc cgt tcc gct ctg cgc ggc   383
Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
            115                 120                 125

ctc cct ccc gtc gac cgc act tac tgt yty gat gg                   418
Leu Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
        130                 135
```

<210> 248
<211> 138
<212> PRT
<213> Hepatitis E virus

<400> 248
Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn Ser
1 5 10 15

Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val Gln
20 25 30

Thr Glu Ile Leu Ile Asn Leu Met Gln Pro Arg Gln Leu Val Phe Arg
35 40 45

Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn Glu
50 55 60

Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly Arg Cys Leu Glu Val Gly
65 70 75 80

Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Leu His Arg Cys
85 90 95

Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro
100 105 110

Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu
115 120 125

Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
130 135

<210> 249
<211> 145
<212> DNA
<213> Hepatitis E virus

<220>
<223> Arl-f73o2p2.pat

<220>
<221> CDS
<222> (1)..(144)

<400> 249
gty gtc tcr gcc aat ggc gag ccg act gtt aag cta tat aca tct gta 48
Xaa Val Xaa Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
1 5 10 15

gag aac gcg cag cag gat aaa ggg atc gcc att cca cac gat ata gat 96
Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
20 25 30

```
ctg ggc gat tcc cgt gtg gtc att cag gat tat gay aac car cay gaa c 145
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
         35                  40                  45


<210> 250
<211> 48
<212> PRT
<213> Hepatitis E virus

<400> 250
Xaa Val Xaa Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
  1               5                  10                  15

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
             20                  25                  30

Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
         35                  40                  45


<210> 251
<211> 418
<212> DNA
<213> Hepatitis E virus

<220>
<223> Ar2ol-f77o1p1.pat

<220>
<221> CDS
<222> (3)..(416)

<400> 251
ct ggc aty act act gcy att gag caa gct gct ctg gct gcg gct aac    47
   Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn
     1               5                  10                  15

tct gcc ttg gcg aat gct gtg gtg gtt cgg ccg ttt cta tcc cgt gtg   95
Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val
                 20                  25                  30

cag act gag atc ctt att aac tta atg car ccc cgg car ctg gtt ttc   143
Gln Thr Glu Ile Leu Ile Asn Leu Met Xaa Pro Arg Xaa Leu Val Phe
             35                  40                  45

cgt ccc gag gtg ctt tgg aat cat ccc att caa cgg gtt att cat aat   191
Arg Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn
         50                  55                  60

gaa tta gag cag tac tgc cgg acc cgg gct ggc cgt tgt tta gag gtc   239
Glu Leu Glu Gln Tyr Cys Arg Thr Arg Ala Gly Arg Cys Leu Glu Val
     65                  70                  75

gga gcc cat cca agg tcc att aat gac aac cct aac gtt cyg cac cgg   287
Gly Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Xaa His Arg
 80                  85                  90                  95
```

```
tgc ttc tta cga cca gtc ggg agg gat gtc caa cga tgg tac tca gcc   335
Cys Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala
                100                 105                 110

ccc act cgc ggc cct gcg gct aat tgc cgt cgt tcc gct ttg cgt ggt   383
Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly
            115                 120                 125

ctc cct cct gtc gac cgc act tac tgt yty gat gg                    418
Leu Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
        130                 135
```

<210> 252
<211> 138
<212> PRT
<213> Hepatitis E virus

<400> 252

```
Gly Xaa Thr Thr Xaa Ile Glu Gln Ala Ala Leu Ala Ala Ala Asn Ser
  1               5                  10                  15

Ala Leu Ala Asn Ala Val Val Val Arg Pro Phe Leu Ser Arg Val Gln
             20                  25                  30

Thr Glu Ile Leu Ile Asn Leu Met Xaa Pro Arg Xaa Leu Val Phe Arg
         35                  40                  45

Pro Glu Val Leu Trp Asn His Pro Ile Gln Arg Val Ile His Asn Glu
     50                  55                  60

Leu Glu Gln Tyr Cys Arg Thr Arg Ala Gly Arg Cys Leu Glu Val Gly
 65                  70                  75                  80

Ala His Pro Arg Ser Ile Asn Asp Asn Pro Asn Val Xaa His Arg Cys
                 85                  90                  95

Phe Leu Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser Ala Pro
            100                 105                 110

Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg Ser Ala Leu Arg Gly Leu
        115                 120                 125

Pro Pro Val Asp Arg Thr Tyr Cys Xaa Asp
    130                 135
```

<210> 253
<211> 197
<212> DNA
<213> Hepatitis E virus

<220>
<223> Ar2o2-f7702.pat

<220>

<221> CDS
<222> (2)..(196)

<400> 253
g aca gaa ttr att tcg tcg gct ggg ggt cag ttg ttt tac tcc cgc cct 49
  Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
    1   5   10   15

gtc gtc tca gcc aat ggc gag ccg act gtt aag ttg tat aca tct gtg   97
Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
             20   25   30

gag aat gcg cag cag gat aaa gga atc gcc atc cca cac gac ata gat   145
Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35   40   45

ctg ggc gat tcc cgt gtg gtt att cag gat tat gay aac car cay gaa   193
Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50   55   60

caa g   197
Gln
 65

<210> 254
<211> 65
<212> PRT
<213> Hepatitis E virus

<400> 254
Thr Glu Xaa Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
  1   5   10   15

Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
             20   25   30

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
         35   40   45

Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Xaa Asn Xaa Xaa Glu
     50   55   60

Gln
 65

<210> 255
<211> 23
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF 1N-a1

<400> 255
ccrtcrarrc artaggtgcg gtc   23

<210> 256
<211> 25
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF 2N-a1

<400> 256
cytgytcrtg ytggttrtca taatc 25

<210> 257
<211> 21
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF 1N-s2

<400> 257
cygccytkgc gaatgctgtg g 21

<210> 258
<211> 25
<212> DNA
<213> Hepatitis E virus

<220>
<223> HEVConsORF 2N-a2

<400> 258
gytcrtgytg rttrtcataa tcctg 25